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(57) Abstract

The present invention provides a method of inhibiting differentiation of hematopoietic progenitor cells by contacting the progenitor cells with an isolated JAGGED polypeptide, or active fragment thereof. The invention additionally provides a method of diagnosing Alagille Syndrome in an individual. The method consists of detecting an Alagille Syndrome disease–associated mutation linked to a JAGGED locus.

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HUMAN JAGGED POLYPEPTIDE, ENCODING NUCLEIC ACIDS AND METHODS OF USE

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BACKGROUND OF THE INVENTION

This invention relates to polypeptides and peptides for regulating stem cell differentiation and renewal and to the molecular defects involved in Alagille Syndrome.

Hematopoiesis involves a delicate balance between progenitor cell self-renewal and differentiation. Self-renewal generates additional progenitor cells through cell division, and differentiation produces specialized cell types such as red blood cells or lymphocytes. The ability to reliably reproduce hematopoietic differentiation and expansion in vitro would greatly facilitate the development of clinical therapeutic treatments based on blood products and cell transplantation. For example, the ability to modulate hematopoietic differentiation and expansion would promote the production of mature blood cells for transfusion therapies and the production of mature dendritic cells for immunotherapy. In addition, the ability to manipulate a hematopoietic cell population to maintain a large number of progenitor cells would greatly improve *ex vivo* retroviral gene therapy since cell proliferation is required for retroviral gene transduction.

The ability to maintain the survival and proliferation of hematopoietic progenitor cells and to inhibit their differentiation would also improve cell transplantation following tumor purging. In high-dose chemotherapy, doses of toxic drugs are escalated to destroy aggressive malignancies such as hematologic, breast, testicular and ovarian cancers. These high doses also destroy many of the rapidly cycling cells of the hematopoietic system, rendering a patient vulnerable to infection. The ability to promote the survival and expansion of a limited number of remaining hematopoietic progenitor cells would increase neutrophil and platelet recovery times and reduce the danger associated with tumor purging and hematopoietic cell transplantation. However, current technology cannot effectively regulate the balance of hematopoietic progenitor cell survival and differentiation.

During embryogenesis in *Drosophila*, the Notch receptor plays a central role in cell fate specification during development of the central and peripheral nervous systems, eye, mesoderm, wing, bristles and ovaries. The Notch family of cell-cell signaling receptors is highly conserved in fly, worm, frog as well as higher vertebrates, and functions to determine cell fate through the transduction of signals between cells in direct contact with each other.

In higher vertebrates, the process of cell-fate determination is integral to hematopoiesis, where the balance between stem cell or progenitor cell self-renewal and differentiation is carefully regulated. Notch homologues can play a role in determining cell fate in hematopoietic cells, as evidenced by the expression of Notch1 RNA in immature hematopoietic precursor cells from adult human bone marrow. Notch homologues are implicated in T lymphocyte development since the human Notch

homologue, TAN-1 (hNotch1), was isolated from a T-cell leukemia containing a translocation between Notch and the T cell receptor (TCR)-β gene. In addition, Notch1 can influence the CD4/CD8 cell-fate decision. Because an activated form of Notch1 can inhibit G-CSF-induced granulocytic differentiation of 32D myeloid progenitors, Notch also can play a role in mediating cell-fate decisions in the myeloid lineage.

The evolutionary conservation of Notch is reflected in the corresponding conservation of Notch ligands. Several Notch ligands have been identified thus far, including Delta and Serrate in *Drosophila*; LAG-2 and APX-1 in *C. elegans*; X-Delta-1 in *Xenopus*; C-Delta-1 and C-Serrate-1 in the chick; Delta-like-1 (Dll1) in the mouse; and Jagged1 and Jagged2 in the rat. Each of these Notch ligands share two important extracellular features: the DSL domain, defined by a conserved region among Delta Serrate, and LAG-2, and tandem epidermal growth factor (EGF) repeats. Delta and Serrate have been shown to interact with Notch in *Drosophila*, and fibroblasts expressing rat Jagged1 inhibit muscle cell differentiation of Notch1-expressing C2C12 cells. These results indicate that DSL family polypeptides including *Drosophila* Delta and Serrate and rat Jagged can function as Notch ligands.

However, a human Notch ligand, which would be useful in manipulating the balance of hematopoietic progenitor cell renewal and differentiation, has not yet been identified. Thus, there is a need for a human Notch ligand and for methods of using the ligand to maintain and expand hematopoietic progenitor cells to make clinical blood products and progenitor cells for transplantation. The present invention satisfies this

need by providing human JAGGED1 polypeptides and provides related advantages as well.

The invention also relates to Alagille Syndrome, which is an autosomal dominant, developmental disorder affecting the liver, heart, skeleton, eye, face and kidneys. The course and prognosis of Alagille Syndrome, which occurs at a minimum estimated frequency of 1 in 70,000 live births, varies widely. This multi-system disorder traditionally has been defined by a paucity of intrahepatic bile ducts in association with several of the main clinical abnormalities, which are cholestasis, cardiac disease, skeletal abnormalities, ocular abnormalities and a characteristic facial phenotype. Fifteen percent of Alagille Syndrome patients will require liver transplantation, and seven to ten percent of patients will have severe congenital heart disease.

Unfortunately, the available therapies for Alagille Syndrome are few, and both diagnosis and treatment have been hampered by a lack of knowledge regarding the molecular defect underlying the disease. In a relatively small number of patients, gross chromosomal deletions of chromosome 20 appear to be inherited with the disorder. However, for the large majority of patients lacking such gross chromosomal abnormalities, the genetic defect responsible for Alagille Syndrome has eluded discovery. Identification of the molecular defect responsible for Alagille Syndrome would be useful in the early diagnosis and prenatal testing of individuals at risk for the disorder. In addition, knowledge of mutations resulting in Alagille Syndrome would facilitate the development of new therapies for treating the disorder. Thus, there is a need for identifying the mutations responsible for Alagille Syndrome and for methods of diagnosing the

disorder by analyzing the genetic defect responsible for the disorder. The present invention satisfies this need and also provides related advantages.

SUMMARY OF THE INVENTION

The present invention provides an isolated polypeptide exhibiting substantially the same amino acid sequence as JAGGED, or an active fragment thereof, provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. The invention further provides an isolated nucleic acid molecule containing a nucleotide sequence encoding substantially the same amino acid sequence as JAGGED, or an active fragment thereof, provided that the nucleotide sequence does not encode the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. Also provided herein is a method of inhibiting differentiation of hematopoietic progenitor cells by contacting the progenitor cells with an isolated JAGGED polypeptide, or active fragment thereof. The invention additionally provides a method of diagnosing Alagille Syndrome in an individual. The method consists of detecting an Alagille Syndrome disease-associated mutation linked to a JAGGED locus.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. (A) Nucleotide sequence SEQ ID NO:1 and amino acid sequence SEQ ID NO:2 of the human JAGGED1 (hJAGGED1) cDNA. (B) Partial nucleotide sequence SEQ ID NO:3 and amino acid sequence SEQ ID NO:4 of the human Jagged 2 (hJAGGED2) cDNA. (C) Diagram showing the protein structure of hJAGGED1 in alignment with the *Drosophila* Delta, *Drosophila* Serrate and rat Jagged1 proteins. The signal peptide region is indicated SP. DSL is a domain unique to Notch ligands, shared by

Drosophila Delta and Serrate and the *C. elegans* protein LAG-2. Also indicated are the epidermal growth factor-like repeats (EGF-like repeats); cysteine-rich region (CR) and transmembrane domain (TM). The percent amino acid identity to hJAGGED1 is shown at the right.

Figure 2. (A) Alignment of hJAGGED1 (hJg1) and rJagged1 (rJg) amino acid sequences. The peptide signal sequence (residues 1 to 21), EGF-like repeats (residues 234 to 862), and transmembrane domain (residues 1077 to 1091) are shown in bold type. The DSL domain (residues 185 to 239) and the cysteine-rich region (residues 863 to 1012) are underlined. (B) Alignment of rat Jagged1 amino acid sequence SEQ ID NO:5 and rat Jagged2 amino acid sequence SEQ ID NO:6.

Figure 3. Inhibition of granulocytic differentiation by the hJAGGED1-expressing stromal cell line, HS-27a. (A) Granulocytic differentiation of 32D myeloid progenitor cells in response to granulocyte colony stimulating factor (G-CSF). The parental 32D cell line (WT) and 32D cells transduced with control LXSN retrovirus or retrovirus containing full-length murine Notch1 cDNA (FL Notch1) were evaluated for granulocytic differentiation in response to G-CSF. The relative percentages of cells remaining undifferentiated (o) or showing morphologic characteristics of mature granulocytes (□) are shown; cells showing some characteristics of differentiation, but which were less mature than band cells were excluded from this analysis. This figure shows results obtained concurrently with those depicted in Figure 4 and represents one of three experiments with comparable results. Plots for the LXSN control clones and the FL Notch1 clones each represent the average obtained for three clones with error bars indicating the SEM. The data for 32D cells expressing the activated Notch1 construct, N1-ICΔOP were obtained on

a separate occasion and represent the averages and SEM of six independent clones. (B) Granulocytic differentiation of 32D cells in the presence of G-CSF when cultured on the human stromal cells line HS-27a, HS-23 or HS-5. The results depicted represent data from three separate experiments, each including three LXSN and three FL Notch1 clones as well as the parental 32D line (not shown). Each plot therefore represents the average and SEM of nine values. The center panels show representative Wright stained cells after four days in culture; the same two clones, LXSN-10 and FLN2.4, are depicted in each set of panels.

Figure 4. Inhibition of granulocytic differentiation by a soluble peptide corresponding to part of the hJAGGED1 DSL domain. 32D clones carrying the control LXSN retroviral vector alone or the vector containing FL Notch1 were evaluated for differentiation in the presence of G-CSF and different peptides corresponding to distinct portions of hJAGGED1. Peptide SEQ ID NO:9 ("J-A") corresponds to a portion of the extracellular DSL domain. Peptide SEQ ID NO:10 ("J-B") corresponds to EGF-repeat 1, and peptide SEQ ID NO:11 ("J-C") corresponds to the intracellular domain. Shown is an experiment using 10 μM peptide. Each plot represents the average and SEM of three independent clones. The center panels show representative Wright stained cells (clones LXSN-10 and FL N 2.4) after 6 days in culture with G-CSF and peptide SEQ ID NO:10 (J-B; top panel) or peptide SEQ ID NO:9 (J-A; lower panel).

Figure 5. Mapping hJAGGED1 in the Alagille Syndrome critical region. The critical region has been defined by the shortest region of overlap of patients with deletions of 20p12 by molecular and FISH mapping and extends between P-1 243b12, proximal to D20S27, and clone 20p1-158, proximal to WI-6063. YAC clones are indicated

in standard print, P1 clones are indicated as such, and BAC clones are in italic print.

Figure 6. (A) Schematic diagram illustrating the alignment of the exon boundaries with the hJAGGED1 cDNA sequence. (B) The exon/intron boundary nucleotide sequences are shown for twenty-four hJAGGED1 exons; sequence identification numbers are indicated in parenthesis. One or more 5' exons have not been identified; the 5' most exon identified to date is indicated exon (n+1). The hJAGGED1 cDNA nucleotide positions corresponding to each exon and the exon length are also indicated.

Figure 7. Heteroduplex Mobility Analysis (HMA) of hJAGGED1 cDNAs in four Alagille Syndrome (AGS) families. (A) A schematic diagram showing the positions of the primers used in RT-PCR, and the amplified cDNA regions A through F. (B) HMA of three members of AGS family 1. PCR product amplified from the hJAGGED1 cDNA clone is shown as a reference (lane J). (C) Analysis of three members of AGS family 2. (D) Analysis of two affected members of AGS family 3 and 4. (E) Analysis of cloned cDNA fragments, each containing one variant. Normal clones from region B, C and D are indicated as B-nl, C-nl and D-nl, respectively. (F) HMA of the hJAGGED1 cDNA region A of 10 individuals from AGS families 1-4, showing no heteroduplex formation.

Figure 8. Segregation of SSCP variants in four Alagille Syndrome families. Individuals with filled circles meet full criteria for diagnosis with Alagille syndrome. Individuals with hatched circles have some of the characteristics of the syndrome. (A) Segregation of an exon (n+2) variant in two children with liver, heart, eye and facial features of Alagille Syndrome and their mildly affected mother. Sequence analysis demonstrates a

2 bp "AG" deletion. (B) Segregation of an exon n+21 variant in a child with Alagille facies and pulmonic stenosis and her more severely affected father. Sequence analysis demonstrates a 5 bp insertion (GTGGC) in father and daughter. (C) Family 3 demonstrates an exon (n+15) variant in an affected mother, her affected daughter and DNA from a terminated pregnancy. Sequence analysis demonstrates a 4 bp deletion in affected individuals. (D) Family 4 has an exon 15 variation in a child with severe cardiac and liver disease who died at 5 years of age and her less severely affected father. Sequence analysis in father and daughter demonstrated a single nucleotide "C" deletion. Sequence identification numbers are indicated in parenthesis.

Figure 9. Summary of the mutations identified in Alagille Syndrome individuals and the corresponding predicted translation products. For each of four Alagille Syndrome mutations, the position of the mutation within the hJAGGED1 cDNA and gene are provided, as well as the predicted amino acid mutations and size of the truncated hJAGGED1 polypeptide.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to the discovery of human Notch ligands, designated JAGGED. The polypeptides of the invention are transmembrane proteins that share several structural features with other Notch ligands, including a DSL (Delta/Serrate/Lag-2) domain characteristic of these ligands and tandem epidermal growth factor (EGF)-like repeats. Provided herein are exemplary JAGGED polypeptides, human JAGGED1 (hJAGGED1) and human JAGGED2 (hJAGGED2). hJAGGED1 is expressed in bone marrow stromal cells, and a stromal cell line expressing hJAGGED1 permits survival and proliferation of hematopoietic progenitor cells expressing Notch but

inhibits granulocytic differentiation. As disclosed herein, a JAGGED-derived peptide can mimic the function of an intact JAGGED molecule by inhibiting the differentiation of Notch-expressing progenitor cells (Example II). Thus, the JAGGED polypeptides and peptides of the invention can be used, for example, in *ex vivo* therapy for inhibiting differentiation and maintaining the proliferative potential of progenitor cells such as hematopoietic stem cells.

Thus, the present invention provides an isolated JAGGED polypeptide. An isolated JAGGED polypeptide of the invention can have substantially the same amino acid sequence as the hJAGGED1 sequence SEQ ID NO:2 shown in Figure 1A or substantially the same amino acid sequence as the hJAGGED2 sequence SEQ ID NO:4 shown in Figure 1B, provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6.

As used herein, the term "JAGGED" means a JAGGED polypeptide and includes polypeptides having substantially the same amino acid sequence as the hJAGGED1 polypeptide (SEQ ID NO:2) shown in Figure 1A or the hJAGGED2 polypeptide (SEQ ID NO:4) shown in Figure 1B, provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. hJAGGED1 exhibits an apparent molecular weight of about 150 kDa on SDS-PAGE and is a 1219 amino acid polypeptide having the sequence shown in Figure 1A. As illustrated in Figure 1C, hJAGGED1 is a membrane-bound ligand with a large extracellular domain and a very short intracellular domain. The hJAGGED1 polypeptide shares structural features with the *Drosophila* polypeptides Delta and Serrate and with the rat Jagged1 polypeptide (see Figure 1C). In particular, hJAGGED1 has a DSL domain, which is a region conserved among the Notch ligands Delta, Serrate and LAG-2. In addition, the extracellular domain of

hJAGGED1 contains EGF repeats. A cysteine-rich domain is also present in hJAGGED1, as in Serrate and rat Jagged1. The DSL and EGF-repeat domains can be involved in interaction with the Notch receptor (Henderson et al., Devel. 120:2913-2924 (1994); Lieber et al., Neuron 9:847-859 (1992); and Rebay et al., Cell 67:687-699 (1991), each of which are incorporated herein by reference).

hJAGGED2 is a polypeptide of more than 1150 amino acids and includes the amino acid sequence shown in Figure 1B. Like hJAGGED1, hJAGGED2 is a membrane-bound ligand with a large extracellular domain and a relatively short intracellular domain. The hJAGGED2 polypeptide also has a DSL domain, 15 EGF-like repeats and a transmembrane domain characteristic of membrane-bound Notch ligands.

As disclosed in Example I, hJAGGED1 is widely expressed in a variety of human tissues. However, in bone marrow, hJAGGED1 expression is restricted to a subpopulation of stromal cells. hJAGGED1 is also expressed in the HS-27a cell line, which is a line of spindle-shaped human stromal cells that do not support differentiation of hematopoietic progenitor cells but support the maintenance of immature progenitors for five to eight weeks. The expression of hJAGGED1 in these cells is consistent with a role for JAGGED polypeptides in regulating hematopoietic progenitor cell survival and differentiation.

Co-culture of myeloid progenitor 32D cells expressing full-length Notch with HS-27a cells, which express hJAGGED1, inhibits G-CSF induced granulocytic differentiation of the 32D cells (see Example II). As disclosed herein, a peptide corresponding to part of the hJAGGED1 DSL domain (residues 188 to 204; SEQ ID NO:9)

also inhibits differentiation of Notch-expressing 32D cells in the presence of G-CSF. Thus, the present invention provides JAGGED polypeptides and peptides useful for maintaining the proliferative potential and inhibiting differentiation of progenitor cells such as hematopoietic progenitor cells.

The term JAGGED encompasses a polypeptide having the sequence of the naturally occurring hJAGGED1 polypeptide (SEQ ID NO:2) or the sequence of the naturally occurring hJAGGED2 polypeptide (SEQ ID NO:4) and is intended to include related polypeptides having substantial amino acid sequence similarity to hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4), provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. Such related polypeptides exhibit greater sequence similarity to hJAGGED1 or hJAGGED2 than to other DSL-containing polypeptides or EGF-repeat containing polypeptides and include alternatively spliced forms of hJAGGED1 or hJAGGED2 and isotype variants of the amino acid sequences shown in Figure 1A and 1B, provided that the polypeptides do not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. The hJAGGED1 and hJAGGED2 polypeptides disclosed herein have about 54% identity to each other at the amino acid level. As used herein, the term JAGGED describes polypeptides generally having an amino acid sequence with greater than about 50% identity, preferably greater than about 60% identity, more preferably greater than about 70% identity, and can be a polypeptide having greater than about 80%, 90%, 95%, 97%, or 99% amino acid sequence identity with hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4), provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6.

A JAGGED polypeptide can be more closely related to hJAGGED1, for example, than to hJAGGED2. Thus, a JAGGED polypeptide can be a member of the JAGGED1 subfamily or a member of the JAGGED2 subfamily. A member of the JAGGED1 subfamily is a polypeptide having substantially the same amino acid sequence as hJAGGED1 (SEQ ID NO:2), or an active fragment thereof, provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5. A member of the JAGGED1 subfamily generally has an amino acid sequence with greater than about 50% identity, preferably greater than about 60% identity, more preferably greater than about 70% identity, and can be a polypeptide having greater than about 80%, 90%, 95%, 97%, or 99% amino acid identity with hJAGGED1 (SEQ ID NO:2), provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5.

Similarly, a member of the JAGGED2 subfamily is a polypeptide having substantially the same amino acid sequence as hJAGGED2 (SEQ ID NO:4), or an active fragment thereof, provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:6. A member of the JAGGED2 subfamily generally has an amino acid sequence with greater than about 50% identity, preferably greater than about 60% identity, more preferably greater than about 70% identity, and can be a polypeptide having greater than about 80%, 90%, 95%, 97%, or 99% amino acid identity with hJAGGED2 (SEQ ID NO:4), provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:6.

As used herein, the term "substantially the same amino acid sequence," when used in reference to a JAGGED amino acid sequence, is intended to mean the sequence shown in Figure 1A or Figure 1B, or a similar, non-identical sequence that is considered by those

skilled in the art to be a functionally equivalent amino acid sequence, provided that the amino acid sequence is not the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. For example, an amino acid sequence that has substantially the same amino acid sequence as JAGGED can have one or more modifications such as amino acid additions, deletions or substitutions relative to the amino acid sequence of hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4), provided that the modified polypeptide retains substantially at least one biological activity of hJAGGED1 or hJAGGED2, such as substantially the ability to bind and activate a Notch receptor or substantially the ability to inhibit progenitor cell differentiation, provided that the modified polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. Comparison of sequences for substantial similarity can be performed between two sequences of any length and usually is performed with nucleotide sequences of between 5 and 3500 nucleotides, preferably between about 10 and 300 nucleotides and more preferably between about 15 and 50 nucleotides. Comparison for substantial similarity between amino acid sequences is usually performed with sequences between about 6 and 1200 residues, preferably between about 10 and 100 residues and more preferably between about 25 and 35 residues. Such comparisons for substantial similarity are performed using methodology routine in the art.

Therefore, it is understood that limited modifications can be made without destroying the biological function of a JAGGED polypeptide and that only a portion of the entire primary sequence can be required in order to effect activity. For example, minor modifications of hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4) that do not destroy polypeptide activity also fall within the definition of JAGGED and within the definition of the polypeptide claimed as such, provided

that such modifications do not produce a polypeptide having the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. Also, for example, genetically engineered fragments of JAGGED either alone or fused to heterologous proteins such as fragments or fusion proteins that retain measurable activity in binding and activating Notch or a Notch homologue, in inhibiting progenitor cell differentiation, or other inherent biological activity of JAGGED fall within the definition of the polypeptide claimed as such.

It is understood that minor modifications of primary amino acid sequence can result in polypeptides which have substantially equivalent or enhanced function as compared to the hJAGGED1 sequence set forth in Figure 1A or the hJAGGED2 sequence set forth in Figure 1B. These modifications can be deliberate, as through site-directed mutagenesis, or can be accidental such as through mutation in hosts harboring a JAGGED encoding nucleic acid. All such modified polypeptides are included in the definition of a JAGGED polypeptide as long as at least one biological function of JAGGED is retained, provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. Further, various molecules can be attached to a JAGGED polypeptide including, for example, other polypeptides, carbohydrates, lipids, or chemical moieties. Such modifications are included within the definition of a JAGGED polypeptide.

Several Notch ligands have been identified including ligands from *Drosophila*, *C. elegans*, *Xenopus*, mouse and rat. Known Notch ligands include Delta and Serrate in *Drosophila* (Baker et al., Science 250:1370-1377 (1990); Cuoso et al., Cell 67:311-323 (1994)); LAG-2 and APX-1 in *C. elegans* (Mello et al.,

Cell 77:95-106 (1994); Tax et al., Nature 368:150-154 (1994); Henderson et al., Develop. 120:2913-2924 (1994)); X-Delta-1 in *Xenopus* (Chitnis et al., Nature 375:761-766 (1995)); C-Delta-1 (Henrique et al., 1995) and C-Serrate-1 in the chick (Myat et al., Dev. Biol. 174:233-247 (1996); Delta-like-1 (Dll1) in the mouse (Bettenhausen et al., Devel. 121:2407-2418 (1995)); and Jagged1 and Jagged2 in the rat (Lindsell et al., Cell 80:909-917 (1995); Shawber et al., Dev. Biol. 370-376 (1996)). However, these Notch ligands are not JAGGED polypeptides as defined herein. The rat Jagged1 polypeptide (SEQ ID NO:5) and rat Jagged2 polypeptide (SEQ ID NO:6) are explicitly excluded from the definition of a JAGGED polypeptide as defined herein. Other Notch ligands described above, which may share the ability to activate Notch or a Notch homologue, lack substantial amino acid sequence similarity with hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4) and, thus, are not JAGGED polypeptides as defined herein.

In one embodiment, the invention provides an isolated JAGGED polypeptide including substantially the same amino acid sequence as JAGGED, or an active fragment thereof, provided that said polypeptide does not have the amino acid sequence of SEQ ID NO:5, the amino acid sequence of SEQ ID NO:6, the amino acid sequence designated by GenBank accession number U61276, the amino acid sequence designated by GenBank accession number U77720, or the amino acid sequence designated by GenBank accession number U77914.

The present invention also provides active fragments of a JAGGED polypeptide. As used herein, the term "active fragment" means a polypeptide fragment having substantially the same amino acid sequence as a portion of a JAGGED polypeptide, provided that the JAGGED

fragment retains at least one biological activity of JAGGED. An active fragment can have, for example, substantially the same amino acid sequence as a portion of hJAGGED1 (SEQ ID NO:2) or substantially the same amino acid sequence as a portion of hJAGGED2 (SEQ ID NO:4). A biological activity of JAGGED can be, for example, the ability to bind and activate Notch or a Notch homologue, the ability to inhibit differentiation of a hematopoietic progenitor cell or the ability to maintain or increase the proliferative potential of a hematopoietic progenitor cell. Examples of active fragments are provided herein as SEQ ID NO:7, which is a soluble active fragment of hJAGGED1 containing residues 1 to 1010, and SEQ ID NO:8, which is a soluble active fragment of hJAGGED1 containing residues 178 to 240. As disclosed in Example II, these soluble JAGGED fragments have activity in inhibiting granulocytic differentiation of primary mouse hematopoietic cells or in increasing their proliferative potential. Explicitly excluded from the definition of an active fragment are polypeptide portions of SEQ ID NO:5 and SEQ ID NO:6.

The term "isolated," as used herein in reference to a polypeptide, peptide or nucleic acid molecule of the invention, means a polypeptide, peptide or nucleic acid molecule that is in a form that is relatively free from contaminating lipids, polypeptides, nucleic acids or other cellular material normally associated with the polypeptide, peptide or nucleic acid molecule in a cell.

A modified JAGGED polypeptide, or fragment thereof, can be assayed for activity using one of the assays described in Example II or using another assay for measuring progenitor cell differentiation or the maintenance of proliferative potential known in the art. For example, a retroviral expression vector containing a

nucleic acid molecule encoding a modified hJAGGED1 or hJAGGED2 polypeptide, or fragment thereof, can be introduced into HS-23 cells, and the transduced cells assayed for the ability to inhibit differentiation of progenitor cells, such as 32D myeloid progenitor cells expressing full-length Notch, in the presence of a differentiating agent such as G-CSF. A soluble JAGGED polypeptide or fragment thereof can be assayed, for example, by introducing an expression vector containing a nucleic acid molecule encoding the soluble JAGGED polypeptide or fragment into a cell and subsequently assaying the culture supernatant for the ability to inhibit hematopoietic progenitor cell differentiation as described in Example II.

The nucleic acid to be assayed can encode an amino acid sequence corresponding to a portion of native hJAGGED1 (SEQ ID NO:2) or native hJAGGED2 (SEQ ID NO:4) or can be modified to encode one or more amino acid substitutions, deletions or insertions, provided that the nucleic acid molecule does not encode the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. One or more point mutations can be introduced into the nucleic acid encoding the modified JAGGED polypeptide or fragment to be assayed using, for example, site-directed mutagenesis (see Wu (Ed.), Meth. In Enzymol. Vol. 217, San Diego: Academic Press (1993); Chapter 22 of Innis et al. (Ed.), PCR Protocols, San Diego: Academic Press, Inc. (1990), each of which is incorporated herein by reference). Such mutagenesis can be used to introduce a specific, desired amino acid substitution, deletion or insertion; alternatively, a nucleic acid sequence can be synthesized having random nucleotides at one or more predetermined positions to generate random amino acid substitutions. Scanning mutagenesis also can be useful in generating nucleic acid molecules encoding JAGGED polypeptides or fragments that are modified throughout the entire

polypeptide or fragment sequence. Such modified fragments can be screened for the ability to inhibit Notch-expressing 32D cell differentiation as described in Example II; for the ability to increase the self-renewal capacity of hematopoietic progenitor cells (Example II); or using another assay for measuring progenitor cell differentiation or the maintenance of progenitor cell proliferative potential that is known in the art.

If desired, a pool of modified JAGGED polypeptides or JAGGED fragments can be assayed for activity *en masse*. For example, to identify an active fragment of hJAGGED1 or hJAGGED2, pools of synthetic JAGGED fragments or pools of cell supernatants can be assayed for the ability to inhibit the differentiation of 32D cells expressing Notch; subsequently, pools of fragments or supernatants with activity can be subdivided, and the assay repeated in order to isolate the active modified hJAGGED1 or hJAGGED2 polypeptide or fragment from the active pool.

An isolated JAGGED polypeptide, or active fragment thereof, can be obtained by a variety of methods known within the art, including biochemical, recombinant and chemical synthesis methods. Biochemical methods for isolating a JAGGED polypeptide, or active fragment thereof, include preparative gel electrophoresis, gel filtration, affinity chromatography, ion exchange and reversed phase chromatography, chromatofocusing, isoelectric focusing and sucrose or glycerol density gradients (see, for example, Chapter 38 of Deutscher, Methods in Enzymology: Guide to Protein Purification, Vol. 182, Academic Press, Inc., San Diego (1990) and Chapter 8 of Balch et al., Methods in Enzymology, Vol. 257, Academic Press, Inc., San Diego (1995), each of which is incorporated herein by reference in its entirety). For example, as disclosed herein in

Example I, hJAGGED1 RNA is expressed in a variety of human tissues, including stomach, thyroid, spinal cord, lymph node, trachea, adrenal gland, and bone marrow, and in the human bone marrow stromal cell line HS-27a (Roecklein and Torok-Storb, Blood 85:997-1005 (1995), which is incorporated herein by reference). From these results, one skilled in the art knows that one of these tissues or the HS-27a cell line can be used as a source of material for isolating a hJAGGED1 polypeptide.

Preparative gel electrophoresis can be useful in preparing an isolated JAGGED polypeptide or active fragment of the invention. For example, a JAGGED polypeptide, or active fragment thereof, can be isolated by preparative polyacrylamide gel electrophoresis and elution of the polypeptide or fragment by diffusion or electroelution (see, for example, Chapter 33 of Deutscher, *supra*, 1990). Continuous elution gel electrophoresis using a system such as the Model 491 Prep Cell (BioRad, Hercules, CA) can be used to purify a JAGGED polypeptide, or active fragment thereof. If desired, continuous elution gel electrophoresis can be combined with further purification steps such as liquid phase preparative isoelectric focusing using, for example, the Rotofor system (BioRad).

Affinity chromatography is particularly useful in preparing an isolated JAGGED polypeptide or active fragment of the invention. A polypeptide that interacts with a JAGGED polypeptide, for example, a Notch polypeptide, can be useful as an affinity matrix for isolating a JAGGED polypeptide or active fragment of the invention. One skilled in the art understands that polypeptide fragments such as fragments of Notch also can be useful affinity matrices for isolating a JAGGED polypeptide or active fragment of the invention.

Immunoaffinity chromatography can be particularly useful in isolating a JAGGED polypeptide or active fragment thereof. For example, immunoprecipitation or column chromatography with an antibody that selectively binds JAGGED can be used to isolate a JAGGED polypeptide or active fragment thereof. An anti-JAGGED monoclonal or polyclonal antibody that selectively binds JAGGED can be prepared using an immunogen such as the sequence shown as SEQ ID NO:2, or a synthetic peptide fragment thereof, as described further below. One skilled in the art understands that a particularly useful immunogen can be a synthetic peptide fragment of SEQ ID NO:2 or SEQ ID NO:4 having a sequence that is relatively unique to JAGGED. Thus, in selecting an immunogen, one can exclude, if desired, regions of SEQ ID NO:2 or SEQ ID NO:4 which are conserved among other proteins. Methods of affinity chromatography are well known in the art and are described, for example, in Chapters 29, 30 and 38 of Deutscher, *supra*, 1990, which has been incorporated herein by reference.

Recombinant methods for producing a polypeptide through expression of a nucleic acid sequence in a suitable host cell also are well known in the art and are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed, Vols 1 to 3, Cold Spring Harbor Laboratory Press, New York (1989), which is incorporated herein by reference. Nucleic acids for expression of a JAGGED polypeptide are provided herein as SEQ ID NO:1 and SEQ ID NO:3. The production of recombinant hJAGGED1 polypeptide is illustrated in Example II.

A recombinant JAGGED polypeptide or active fragment of the invention can be expressed as a fusion protein with a heterologous "tag" for convenient

isolation from bacterial or mammalian host proteins. For example, histidine-tagged recombinant JAGGED can be isolated by nickel-chelate chromatography. Similarly, a glutathione-S-transferase tag or an antigenic tag such as "FLAG," "AU" or a myc epitope tag also can be included in a recombinant JAGGED polypeptide or active fragment of the invention (Sambrook et al., *supra*, 1989). The use of the PinPoint™ expression system for expression of the hJAGGED1 active fragment SEQ ID NO:8 as a fusion protein with a heterologous biotinylated peptide is illustrated in Example II.

A JAGGED polypeptide fragment or a JAGGED peptide of the invention can be produced by chemical synthesis, for example, by the solid phase peptide synthesis method of Merrifield et al., J. Am. Chem. Soc. 85:2149 (1964), which is incorporated herein by reference. Standard solution methods well known in the art also can be used to synthesize a peptide or polypeptide fragment useful in the invention (see, for example, Bodanszky, Principles of Peptide Synthesis, Springer-Verlag, Berlin (1984) and Bodanszky, Peptide Chemistry, Springer-Verlag, Berlin (1993), each of which is incorporated herein by reference). A newly synthesized peptide or polypeptide fragment can be purified, for example, by high performance liquid chromatography (HPLC) and can be characterized using mass spectrometry or amino acid sequence analysis.

A JAGGED polypeptide of the invention is useful for preparing an antibody that selectively binds a JAGGED polypeptide such as hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4). An antibody that selectively binds a JAGGED polypeptide can be useful, for example, in purifying a JAGGED polypeptide by immunoaffinity chromatography. Such an antibody also can be useful in diagnosing Alagille Syndrome in an individual by

detecting reduced expression of a JAGGED polypeptide or by detecting an abnormal JAGGED gene product such as a truncated hJAGGED1 gene product. A particularly useful diagnostic antibody can be, for example, an antibody that selectively binds a C-terminal epitope of hJAGGED1, such that the amount of full-length hJAGGED1 polypeptide in a sample can be analyzed.

As used herein, the term antibody is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as polypeptide fragments of antibodies that retain selective binding activity for a JAGGED polypeptide of at least about 1×10^5 M^{-1}. One skilled in the art would know that anti-JAGGED antibody fragments such as Fab, $F(ab')_2$ and Fv fragments can retain selective binding activity for a JAGGED polypeptide and, thus, are included within the definition of an antibody. In addition, the term antibody as used herein includes naturally occurring antibodies as well as non-naturally occurring antibodies and fragments that have binding activity such as chimeric antibodies or humanized antibodies. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis or produced recombinantly. Such non-naturally occurring antibodies also can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Borrebaeck (Ed.), Antibody Engineering (Second edition) New York: Oxford University Press (1995), which is incorporated herein by reference.

An antibody selective for a polypeptide, or that selectively binds a polypeptide, binds with substantially higher affinity to that polypeptide than to an unrelated polypeptide. An antibody selective for a polypeptide also can be selective for a related polypeptide. For example, an antibody selective for

human JAGGED1 (SEQ ID NO:2) also can be selective for hJAGGED2 (SEQ ID NO:4) or for JAGGED1 homologs from other species.

An anti-JAGGED antibody can be prepared, for example, using a JAGGED fusion protein or a synthetic peptide encoding a portion of JAGGED such as hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4) as an immunogen. One skilled in the art would know that a purified JAGGED polypeptide, which can be prepared from natural sources or produced recombinantly as described above, or fragments of JAGGED, including a peptide portion of JAGGED such as a synthetic peptide, can be used as an immunogen. Non-immunogenic fragments or synthetic peptides of JAGGED can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). In addition, various other carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art and described, for example, by Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1988), which is incorporated herein by reference.

The present invention also provides an isolated nucleic acid molecule that contains a nucleotide sequence encoding substantially the same amino acid sequence as JAGGED, or an active fragment thereof, provided that the nucleic acid molecule does not encode the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. An isolated nucleic acid molecule of the invention can have a nucleotide sequence encoding the same amino acid sequence as hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4) or can encode an amino acid sequence with substantial similarity to SEQ ID NO:2 or SEQ ID NO:4, provided that the nucleic acid molecule does not encode the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. An isolated

nucleic acid molecule of the invention can have, for example, a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. Such isolated nucleic acid molecules are exemplified herein as SEQ ID NO:1 and SEQ ID NO:3.

In one embodiment, the invention provides an isolated nucleic acid molecule that contains a nucleotide sequence encoding substantially the same amino acid sequence as JAGGED, or an active fragment thereof, provided that nucleic acid sequence does not encode the amino acid sequence of SEQ ID NO:5, the amino acid sequence of SEQ ID NO:6, the amino acid sequence designated by GenBank accession number U61276, the amino acid sequence designated by GenBank accession number U77720, or the amino acid sequence designated by GenBank accession number U77914.

As used herein, the term "isolated nucleic acid molecule" means a nucleic acid molecule that is in a form that is relatively free from contaminating lipids, polypeptides, unrelated nucleic acids and other cellular material normally associated with a nucleic acid molecule in a cell.

An isolated nucleic acid molecule of the invention can be, for example, a nucleic acid molecule encoding an alternatively spliced JAGGED variant, a polymorphic variant, a nucleic acid molecule that is related, but different, and encodes thé same JAGGED polypeptide due to the degeneracy of the genetic code, or a nucleic acid molecule that is related, but different and encodes a different JAGGED polypeptide that exhibits at least one biological activity of JAGGED, provided that the nucleic acid molecule does not encode the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6.

The present invention also provides a cell containing a recombinant nucleic acid molecule having a nucleotide sequence encoding substantially the same amino acid as JAGGED, or active fragment thereof, provided that the nucleotide sequence does not encode the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. The encoded JAGGED polypeptide can be, for example, hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4), or an active fragment thereof, including soluble active fragments and membrane-bound active fragments. The cell can be a prokaryotic cell or a eukaryotic cell such as an HS-23 human stromal cell, COS cell or BHK cell.

An HS-23 cell can be particularly useful for expressing a recombinant nucleic acid molecule encoding a membrane-bound form of a JAGGED polypeptide. HS-23 cells can be transduced with retroviral vectors to express membrane-bound JAGGED variants and can be used as a stromal cell layer for maintaining hematopoietic progenitor cells and inhibiting their differentiation. As described in Example II, a COS or BHK cell can be particularly useful for expressing a recombinant nucleic acid molecule encoding a soluble form of JAGGED, such as an active fragment having hJAGGED1 amino acids 1 to 1010 (SEQ ID NO:7) or an active fragment having hJAGGED1 amino acids 178 to 240 (SEQ ID NO:8). The supernatant from such a COS or BHK cell has the activity of the soluble active JAGGED fragment and can be used in crude form to inhibit the differentiation of hematopoietic progenitor cells or as a source for purifying the soluble active JAGGED fragment.

The present invention also provides an isolated JAGGED peptide having at most about 40 amino acids and including substantially the same amino acid sequence as SEQ ID NO:9. A JAGGED peptide of the invention can be, for example, a peptide of up to about forty amino acids

including the amino acid sequence SEQ ID NO:9, or a substantially similar sequence. A JAGGED peptide can have, for example, about 20, 25, 30, 35 or 40 amino acids including the amino acid sequence of SEQ ID NO:9 or a substantially similar sequence. Provided herein is an example of an isolated JAGGED peptide, which has the amino acid sequence Cys-Asp-Asp-Tyr-Tyr-Tyr-Gly-Phe-Gly-Cys-Asn-Lys-Phe-Cys-Arg-Pro-Arg (SEQ ID NO:9).

The JAGGED peptide SEQ ID NO:9 has the amino acid sequence of residues 188 to 204 of hJAGGED1, which corresponds to a portion of the conserved DSL domain. As disclosed herein, this 17-mer peptide SEQ ID NO:9 can mimic the function of hJAGGED1 in promoting survival and inhibiting differentiation of Notch-expressing myeloid progenitor cells in the presence of a differentiating stimulus. Figure 4 shows that differentiation of 32D clones expressing Notch1 was unaffected by treatment with peptide SEQ ID NO:10 ("J-B") or SEQ ID NO:11 ("J-C"). However, differentiation was significantly inhibited in the presence of the JAGGED peptide SEQ ID NO:9 ("J-A") as shown in the lower right panel of Figure 4. This inhibition was similar to that observed when Notch-expressing 32D cells were cultured with hJAGGED1-expressing HS-27a stromal cells. Thus, a JAGGED peptide of the invention has activity in inhibiting the differentiation of progenitor cells and can be useful, for example, in the *in vitro* expansion of a variety of hematopoietic progenitor cell types.

The present invention therefore provides methods of using the JAGGED polypeptides and peptides of the invention. The present invention provides a method of inhibiting differentiation of hematopoietic progenitor cells by contacting the hematopoietic progenitor cells with an isolated JAGGED polypeptide having substantially the same amino acid sequence as JAGGED, or an active

fragment thereof. An isolated JAGGED polypeptide useful in the methods of the invention can have substantially the same amino acid sequence as hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4) or can be an active fragment.

The invention also provides a method of inhibiting differentiation of progenitor cells by contacting the progenitor cells with an isolated JAGGED peptide having at most about forty amino acids and including substantially the same amino acid sequence as SEQ ID NO:9. Such progenitor cells can be hematopoietic progenitor cells and can be contacted, for example, *in vitro*. Such an isolated JAGGED peptide of the invention can be, for example, a peptide of up to about forty amino acids which includes the amino acid sequence SEQ ID NO:9 or a substantially similar sequence. For example, an isolated JAGGED peptide useful in the methods of the invention can be a peptide having the sequence Cys-Asp-Asp-Tyr-Tyr-Tyr-Gly-Phe-Gly-Cys-Asn-Lys-Phe-Cys-Arg-Pro-Arg (SEQ ID NO:9).

As used herein, the term "progenitor cell" means any cell capable of both self-renewal and differentiation. Thus, a progenitor cell can proliferate under appropriate conditions to produce an increased number of progenitor cells, or can differentiate under appropriate conditions to produce cells of specialized function. A progenitor cell can be a committed or unipotent progenitor cell that differentiates into one particular differentiated cell type. A progenitor cell also can be a pluripotent progenitor cell that has the potential to differentiate into multiple different cell types. A progenitor cell can be, for example, a hematopoietic progenitor cell, a neuronal precursor cell, a muscle progenitor cell, a hepatic progenitor cell or another cell capable of both self-renewal and differentiation. One skilled in the art understands that

a progenitor cell useful in the invention expresses a JAGGED receptor, which can be, for example, a Notch polypeptide.

The term "hematopoietic progenitor cell," as used herein, means a progenitor cell capable of differentiating to one or more red or white blood cell types. A hematopoietic progenitor cell can be, for example, a totipotent hematopoietic stem cell capable of both self-renewing and differentiating to all hematopoietic cell types, thereby producing erythrocytes, granulocytes, monocytes, mast cells, lymphocytes and megakaryocytes. A hematopoietic progenitor cell also can be, for example, a lymphoid progenitor or myeloid progenitor cell. A lymphoid progenitor cell generates T and B progenitor lymphocytes. A myeloid progenitor cell generates progenitor cells for erythrocytes, neutrophils, eosinophils, basophils, monocytes, mast cells and platelets. In nature, bone marrow stromal cells produce the membrane-bound and diffusible factors responsible for maintaining an appropriate balance between hematopoietic progenitor cell proliferation and differentiation.

The present invention provides methods of maintaining progenitor cells in an undifferentiated state by contacting progenitor cells with a JAGGED polypeptide, or active fragment thereof. The progenitor cells can be cells capable of reconstituting the hematopoietic system such as hematopoietic stem cells. In one embodiment, the progenitor cells are maintained in a totipotent state capable of differentiating into all the specialized cell types of the hematopoietic system.

Subsequent to treating progenitor cells according to a method of the invention, the progenitor cells can be subject to cryopreservation, for example, by freezing in liquid nitrogen and can be stored, if

desired, for a period of months, years or decades and later thawed for further expansion or differentiation. Thus, progenitor cells can be obtained from a newborn, for example, "locked" into an undifferentiated state using a JAGGED polypeptide according to a method of the invention, and stored for future use for an indefinite period.

The methods of the invention also represent advances in cell transplantation and gene therapy. In one embodiment, progenitor cells maintained in an undifferentiated state according to the methods of the invention can be subsequently transplanted into an individual, such that the progenitor cells differentiate fully in the individual. The progenitor cells can be, for example, totipotent hematopoietic stem cells, which differentiate fully in the individual to reconstitute the hematopoietic system.

The methods of the invention therefore have utility in cell transplantation, including bone marrow transplantation, peripheral blood stem cell transplantation and umbilical cord blood transplantation (McAdams et al., Trends in Biotech. 14:341-349 (1996), which is incorporated herein by reference). The cell transplantation methods of the invention can be useful, for example, in replacing the hematopoietic stem cells of a cancer patient having a leukemia or lymphoma such as acute myelogeous leukemia (AML), non-Hodgkin's lymphoma or chronic myelogenous leukemia.

The progenitor cells can be, for example, autologous or allogeneic to the individual into which the transplanted cells are introduced. When the progenitor cells are derived from a cancer patient, the progenitor cells can be obtained by purging bone marrow or peripheral blood with, for example, chemical agents,

immunomagnetic beads, antisense oligonucleotides or antibodies. If desired, progenitor cells can be sorted prior to treating with a JAGGED polypeptide, or active fragment thereof, according to a method of the invention. For example, progenitor cells can be sorted to obtain $CD34^+$ stem cells, which are contacted with a JAGGED polypeptide or active fragment thereof to maintain the $CD34^+$ stem cells in an undifferentiated state capable of full differentiation, and subsequently transplanted into an individual such that the $CD34^+$ stem cells differentiate fully and reconstitute the entire hematopoietic system of the individual.

The methods of the invention also have gene therapy applications. A nucleic acid molecule encoding a gene product can be introduced into progenitor cells maintained in an undifferentiated state according to a method of the invention. Gene therapy methods for introducing a nucleic acid molecule into a cell such as a progenitor cell are well known in the art and include retroviral and adenoviral methods as well as liposome-mediated and other gene transfer technologies as described in Chang (Ed), Somatic Gene Therapy Boca Raton, CRC Press, Inc. (1995), which is incorporated herein by reference. The methods of the invention, involving the use of a JAGGED polypeptide or JAGGED peptide for maintaining progenitor cells in an undifferentiated state, are particularly useful when combined with retroviral gene transfer methods, which require that cells be in a proliferating state.

The invention also provides a method of maintaining progenitor cells in an undifferentiated state by contacting the progenitor cells with a JAGGED peptide having at most about 40 amino acids and containing substantially the same amino acid sequence as SEQ ID NO:9. In the methods of the invention, the progenitor

cells can be capable of reconstituting the hematopoietic system. The progenitor cells can be maintained in a totipotent state and can be, for example, maintained in culture.

The invention further provides a method of maintaining progenitor cells in an undifferentiated state by contacting the progenitor cells with a JAGGED peptide having at most about 40 amino acids and containing substantially the same amino acid sequence as SEQ ID NO:9 and cryopreserving the progenitor cells maintained in an undifferentiated state. In addition, the invention provides a method of maintaining progenitor cells in an undifferentiated state by contacting the progenitor cells with a JAGGED peptide having at most about 40 amino acids and containing substantially the same amino acid sequence as SEQ ID NO:9 and introducing a nucleic acid molecule encoding a gene product into the progenitor cells.

The JAGGED polypeptides, active fragments and JAGGED peptides of the invention can be administered in a variety of dosage regimes to modulate the inhibitory effect on undifferentiated hematopoietic progenitor cells. For example, a JAGGED polypeptide, active fragment or JAGGED peptide can be administered in a single bolus of an effective concentration, or alternatively, multiple treatments of a JAGGED polypeptide, active fragment or JAGGED peptide can be administered to, for example, modulate or enhance the inhibitory effect on hematopoietic progenitor cells. Similarly, the amount of a JAGGED polypeptide, active fragment or JAGGED peptide that is administered can be increased or decreased so as to modulate the inhibitory effect on hematopoietic progenitor cell differentiation. A JAGGED polypeptide, active fragment or JAGGED peptide also can be administered in combination with other compounds which can modulate hematopoietic cell

differentiation or can modulate other therapeutic events. Such procedures are known to those skilled in the art.

The inhibition of hematopoietic progenitor cell differentiation also can be modulated by altering the activity of a JAGGED polypeptide receptor. Activity can be altered by, for example, increasing the amount or expression level of a JAGGED polypeptide or by modulating the activation of a JAGGED receptor. Other methods exist as well and are known or can be determined by those skilled in the art.

As disclosed herein, molecular defects in hJAGGED1 can cause Alagille Syndrome, which is an autosomal dominant, developmental disorder that affects structures in the liver, heart, skeleton, eye, face, kidney and other organs. The minimal estimated frequency of the syndrome is 1 in 70,000 live births. The syndrome traditionally has been defined by a paucity of intrahepatic bile ducts in association with several of the main clinical abnormalities: cholestasis, cardiac disease, skeletal abnormalities, ocular abnormalities and a characteristic facial phenotype. Cholestasis occurs as a consequence of the paucity of bile ducts. Cardiac anomalies most commonly involve the peripheral and main pulmonary arteries as well as the pulmonary valves. The most common skeletal anomalies are "butterfly" or hemivertebrae, resulting from clefting abnormalities of the vertebral bodies. Ocular lesions include anterior chamber defects, most commonly posterior embryotoxon, which is a benign defect, and retinal pigmentary abnormalities. Facies have been described as triangular, consisting of a prominent forehead, deep-set eyes, hypertelorism, long straight nose with flattened tip, short philtrum, flat midface and a triangular chin. Renal and neurodevelopmental abnormalities occur less frequently. Fifteen percent of patients will require

liver transplantation and seven to ten percent of patients have severe congenital heart disease, most often tetralogy of Fallot (Walker et al. (Eds), Gastrointestinal Disease: Pathophysiology, Diagnosis, Management (3rd edition) B.C. Decker, Inc., Philadelphia pp 1124-1140 (1991), which is incorporated herein by reference). An Alagille Syndrome diagnosis is made if bile duct paucity is accompanied by three of the five main clinical criteria. The expressivity of Alagille Syndrome is variable; accordingly, family members of a proband are considered affected if they express any of the five main clinical features.

The genetic defect underlying this multi-system disorder has been mapped to a 1.5 Mb segment based on analysis of overlapping chromosomal deletions at 20p11-12. Identified herein is the gene responsible for the Alagille Syndrome disorder, the human Notch ligand, hJAGGED1. Four distinct coding region mutations in the hJAGGED1 gene were identified and shown to segregate with disease phenotype in four Alagille Syndrome families. As disclosed in Example V and summarized in Figure 9, all four mutations lie within conserved regions of the hJAGGED1 gene: within the DSL domain, the EGF-repeats and the cysteine-rich region. Each of these mutations are predicted to produce a translational frameshift resulting in a gross alteration of the hJAGGED1 gene product. Furthermore, none of the mutations observed in Alagille Syndrome families were present in 100 normal control chromosomes studied. Thus, from the hundreds of potential genes within the cytogenetic deletion 20p11-12, the hJAGGED1 gene product has been identified as responsible for Alagille Syndrome. Based on this identification, the present invention provides methods of diagnosing Alagille Syndrome in a individual. Such methods can be useful in the early diagnosis or prenatal testing of individuals at risk for the disorder and can

facilitate the development of therapies for affected individuals.

The present invention provides a method of diagnosing Alagille Syndrome in an individual by detecting a disease-associated mutation linked to a JAGGED locus. The disease-associated mutation can be linked but outside a JAGGED gene or can be within a JAGGED gene, for example, in a JAGGED coding sequence, 5' or 3' regulatory region, or within an intronic sequence.

In one embodiment of the invention, the JAGGED locus is a human JAGGED1 (hJAGGED1) locus. In the methods of the invention, the disease-associated mutation can produce, for example, an inactive hJAGGED1 gene product such as a truncated hJAGGED1 gene product. Examples of Alagille Syndrome disease-associated mutations occurring within the hJAGGED1 nucleotide sequence SEQ ID NO:1 are provided herein and include nucleotide variations at nucleotides 1104-1105, nucleotide 3102, nucleotides 2531-2534 and nucleotide 2066 of SEQ ID NO:1.

As used herein, the term "linked" means that two genetic loci have a tendency to be inherited together as a result of their proximity. If two genetic loci are linked and are polymorphic, one locus can serve as a marker for the inheritance of the second locus. Thus, an Alagille Syndrome disease-associated mutation linked to a JAGGED locus having a modified JAGGED allele causing Alagille Syndrome can serve as a marker for inheritance of the modified JAGGED allele. Such a linked mutation can be located in proximity to a JAGGED gene or can be located within a JAGGED gene.

The term "JAGGED locus," as used herein, means a locus encoding a JAGGED gene product. A JAGGED locus can be, for example, the human JAGGED1 locus, positioned within markers D20S894 and D20S507, as described in Example III.

The term "Alagille Syndrome disease-associated mutation," as used herein, is synonymous with "disease-associated mutation" and means a molecular variation of at most several thousand nucleotides that tends to be inherited together with the Alagille Syndrome disorder.

Disclosed herein are a variety of Alagille Syndrome disease-associated mutations linked to the hJAGGED1 locus. Distinct disease-associated mutations, which occur within the hJAGGED1 coding sequence, were found in each of four Alagille Syndrome families as summarized in Figure 9. In a first Alagille Syndrome family, a deletion of "AG" at positions 1104-1105 of SEQ ID NO:1 produced a protein truncated at amino acid 240. In a second family, an insertion of five nucleotides ("GTGGC") at position 3102 of SEQ ID NO:1 produced a protein truncated at amino acid 945, while in a third family, a deletion of "CAGT" at positions 2531-2534 of SEQ ID NO:1 resulted in a protein truncated at amino acid 741. In a fourth Alagille Syndrome family, a single "C" nucleotide deletion at position 2066 of SEQ ID NO:1 resulted in a protein truncated at amino acid 563.

A disease-associated mutation useful in diagnosing Alagille Syndrome can be, for example, a nucleotide substitution, insertion or deletion of one or more nucleotides that tends to be inherited together with Alagille Syndrome. For example, the molecular variation can be a nucleotide substitution, insertion or deletion of about 1 to 3000 nucleotides, such as a substitution,

insertion or deletion of about 1 to 1000 nucleotides, about 1 to 100 nucleotides, about 1 to 50 nucleotides or about 1 to 10 nucleotides. Disclosed herein are a two nucleotide deletion, five nucleotide insertion, four nucleotide deletion and single nucleotide deletion, which are mutations associated with Alagille Syndrome (Example V). One skilled in the art understands that a disease-associated mutation also can be a molecular variation such as abnormal methylation or other modification that does not produce a difference in the primary nucleotide sequence of the disease-associated allele as compared to the normal allele. Specifically excluded from the definition of an Alagille Syndrome disease-associated mutation are large nucleotide variations of more than several thousand nucleotides, including gross cytogenetic deletions and megabase deletions such as those reported in Rand et al., Am. J. Hum. Genet. 57:1068-1073 (1995), which is incorporated herein by reference.

An Alagille Syndrome disease-associated mutation can occur within a JAGGED gene and can result, for example, in production of an inactive JAGGED gene product or a reduced amount of a JAGGED gene product. For example, an Alagille Syndrome disease-associated mutation within a JAGGED gene can be a nucleotide modification within a gene regulatory element such that a JAGGED gene product is not produced or a nucleotide modification within an intronic sequence resulting in an abnormally spliced, inactive JAGGED gene product. In addition, an Alagille Syndrome disease-associated polymorphism can be a nucleotide modification resulting in one or more amino acid substitutions, deletions or insertions in a JAGGED coding sequence, which result in an inactive JAGGED gene product. For example, an inactive JAGGED gene product can result from a frameshift or nonsense mutation producing a truncated JAGGED gene

product, a missense mutation, or a gross nucleotide insertion or deletion. Such an inactive JAGGED gene product can be, for example, a JAGGED polypeptide variant lacking the ability to activate Notch or a soluble JAGGED polypeptide that functions as a dominant negative molecule when expressed with wild type JAGGED polypeptide or another JAGGED polypeptide variant lacking one or more biological functions of JAGGED.

A variety of molecular methods useful in detecting an Alagille Syndrome disease-associated mutation linked to a JAGGED locus are well known in the art. For example, allele-specific oligonucleotide hybridization involves the use of a labeled oligonucleotide probe having a sequence perfectly complementary, for example, to a disease-associated sequence. Under appropriate conditions, the allele-specific probe hybridizes to a nucleic acid containing the disease-associated mutation but does not hybridize to the corresponding wild type nucleic acid sequence having one or more nucleotide mismatches. If desired, a second allele-specific oligonucleotide probe that matches the wild type sequence also can be used. Similarly, the technique of allele-specific oligonucleotide amplification can be used to selectively amplify, for example, a disease-associated polymorphic sequence by using an allele-specific oligonucleotide primer that is perfectly complementary to the nucleotide sequence of a disease-associated allele but which has one or more mismatches as compared to the corresponding wild type sequence (Mullis et al. (Eds.), The Polymerase Chain Reaction, Birkhäuser, Boston, (1994), which is incorporated herein by reference). Particularly useful allele-specific oligonucleotides are oligonucleotides that correspond to about 15 to about 40 nucleotides of the hJAGGED1 nucleotide sequence SEQ ID NO:1 and that include one of the disease-associated polymorphic regions

identified herein: nucleotides 1104-1105, nucleotide 3102, nucleotides 2531-2534 or nucleotide 2066 of SEQ ID NO:1. One skilled in the art understands that the one or more nucleotide mismatches that distinguish between the disease-associated and wild type allele are preferably located in the center of an allele-specific oligonucleotide primer to be used in allele-specific oligonucleotide hybridization. In contrast, an allele-specific oligonucleotide primer to be used in PCR amplification preferably contains the one or more nucleotide mismatches that distinguish between the disease-associated and wild type alleles at the 3' end of the primer.

A heteroduplex mobility assay (HMA) is another well known assay that can be used to diagnose Alagille Syndrome according to a method of the invention. HMA is useful for detecting the presence of a polymorphic sequence since a DNA duplex carrying a mismatch, such as a heteroduplex between a wild type and mutated DNA fragment, has reduced mobility in a polyacrylamide gel compared to the mobility of a perfectly base-paired duplex (Delwart et al., Science 262:1257-1261 (1993); White et al., Genomics 12:301-306 (1992), each of which is incorporated herein by reference). Methods for detecting an Alagille Syndrome disease-associated mutation using a heteroduplex mobility assay are set forth in Example V.

The technique of single strand conformation polymorphism (SSCP) also can be used to detect the presence of an Alagille Syndrome disease-associated mutation (see Hayashi, PCR Methods Applic. 1:34-38 (1991), which is incorporated herein by reference). This technique can be used to detect mutations based on differences in the secondary structure of single-strand DNA that produce an altered electrophoretic mobility upon

non-denaturing gel electrophoresis. Polymorphic fragments are detected by comparison of the electrophoretic pattern of the test fragment to the corresponding fragment from a normal individual of a non-Alagille Syndrome family. The detection of an Alagille Syndrome disease-associated mutation using SSCP is exemplified in Example V.

Denaturing gradient gel electrophoresis (DGGE) also can be used to detect an Alagille Syndrome disease-associated mutation linked to a JAGGED locus. In DGGE, double-stranded DNA is electrophoresed in a gel containing an increasing concentration of denaturant; double-stranded fragments made up of mismatched wild type and disease-associated sequences have segments that melt more rapidly, causing such fragments to migrate differently as compared to perfectly complementary sequences obtained from normal individuals (Sheffield et al., "Identifying DNA Polymorphisms by Denaturing Gradient Gel Electrophoresis" in Innis et al., *supra*, 1990).

Other well-known approaches for analyzing a mutation include automated sequencing, RNAase mismatch techniques (Winter et al., Proc. Natl. Acad. Sci. 82:7575-7579 (1985), which is incorporated herein by reference) and the use of restriction fragment length polymorphisms (see Innis et al., *supra*, 1990). For families in which the disease-associated mutation has been defined, automated sequencing of the region of interest can be particularly useful in diagnosing Alagille Syndrome. Thus, the methods of the invention for diagnosing Alagille Syndrome in an individual can be practiced using a heteroduplex mobility assay or single strand conformation polymorphism assay as illustrated in Example V, using one of the well known assays described

above, or another art-recognized assay for detecting a disease-associated mutation.

The present invention also relates to the presence of genetic polymorphisms in human JAGGED2 and their association with a human syndrome characterized by syndactyly and cleft palate or lip. As disclosed herein, the hJAGGED2 gene can be responsible for the developmental abnormalities in patients with syndactyly, with cleft palate or lip, or with both syndactyly and cleft palate or lip.

Thus, the present invention provides a method of diagnosing a syndrome characterized by syndactyly and cleft palate or lip in a human, comprising detecting a syndactyly and cleft palate or lip-associated mutation linked to a human JAGGED2 locus. In such a method, the syndrome-associated mutation can be within a hJAGGED2 locus, for example, within a hJAGGED2 regulatory element or coding sequence. A syndrome associated mutation can produce, for example, a point mutation or truncation that alters the expression or activity of hJAGGED2.

A mutation associated with a syndrome characterized by syndactyly and cleft palate or lip can be detected by a variety of methodologies including, for example, allele-specific oligonucleotide hybridization, denaturing gradient gel electrophoresis, heteroduplex mobility assays, single strand conformation polymorphism assays, automated sequencing, RNAase mismatch techniques, or restriction fragment length polymorphism-based approaches, as described above in regard to the detection of mutations associated with Alagille Syndrome. The skilled person will recognize that a syndactyly and cleft palate or lip-associated mutation can be detected with these or other routine methodologies known in the art of genetics.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

ISOLATION AND CHARACTERIZATION OF HUMAN JAGGED1

This example describes the isolation, characterization and expression of human JAGGED1.

Isolation of the Human JAGGED1 cDNA

A cDNA encoding a human Notch ligand expressed in the bone marrow microenvironment was isolated by amplifying human bone marrow cDNA with degenerate primers SEQ ID NO:12 and SEQ ID NO:13, which correspond to portions of the conserved DSL and EGF-like repeat domains of rat Jagged1 (rJagged; Lindsell et al., *supra*, 1995). Ten PCR products of potential interest were identified, cloned and sequenced. The clone Sdi-06 contains a 327 bp insert that encodes part of the DSL and EGF-repeat domains. The sequence of this fragment has 96% predicted amino acid sequence identity with the corresponding region of rJagged1 (residues 205 to 312), 84% predicted amino acid sequence identity with C-Serrate-1 (residues 178-286), and 52% predicted amino acid sequence identity with C-Delta-1 (residues 203-311). Thus, the Sdi-06 clone encodes a partial cDNA fragment of the human homolog of rJagged1.

The complete hJAGGED1 cDNA was obtained by screening a human bone marrow cDNA library with ^{32}P-labeled Sdi-06. One of the cDNA clones isolated, D-01, was found to contain the 5'-end of human JAGGED1 including 417 bp of 5' untranslated sequence and 2270 bp of coding sequence. The 3' end of hJAGGED1 was obtained

by rescreening the same human bone marrow cDNA library with ^{32}P-labeled rat Jagged1 cDNA provided by Dr. Weinmaster (Lindsell et al., *supra*, 1995). A cDNA clone identified with this probe, designated Y-A01, contains 2.4 kb of coding region and 1.5 kb of 3' untranslated region. A full-length 5.5 kb hJAGGED1 cDNA was assembled from the 5' D-01 clone and the 3' Y-A01 clone as described further below.

The full-length hJAGGED1 clone has an open reading frame of 3657 base pairs and encodes a predicted protein product of 1219 amino acids (Figure 1A). Analysis of the amino acid sequence indicates that hJAGGED1 is a transmembrane protein with a large extracellular domain and a very short intracellular domain. The hJAGGED1 protein shares structural features with the *Drosophila* Notch ligands Delta and Serrate and with rat Jagged1. The shared structural features include a DSL motif and 16 epidermal growth factor-like (EGF-like) repeats within the extracellular domain. A cysteine-rich region present in Serrate and rJagged1 is also conserved in hJAGGED1 (Figure 1C).

An alignment of the amino acid sequences of hJAGGED1 (hjg) and rJagged1 (rjg) is shown in Figure 2A. The hJAGGED1 protein has 94% overall amino acid identity with rJagged1, with 96% amino acid identity with the highly conserved DSL and EGF-repeat domains. Several distinctive amino acid substitutions are present in the hJAGGED1 sequence relative to rJagged1. Two prolines in the signal peptide region of rJagged1 are replaced with arginine and serine in hJAGGED1 (residues five and ten, respectively). In addition, the region between the signal peptide and the DSL motif is dissimilar (compare residues 56 to 64 in hJAGGED1 (GGARNPGDR; SEQ ID NO:14) to residues 56 to 65 in rJagged1 (AEPGTLVRPY; SEQ ID NO:15). Other amino acid differences include a proline

to phenylalanine substitution within the DSL motif (residue 194 of hJAGGED1); amino acid differences within the EGF-repeat region; and a serine to cysteine substitution within the cysteine-rich domain, (residue 860 of hJAGGED1). In the intracellular domain, a proline to serine substitution occurs at residue 1107 of hJAGGED1, and a valine to proline substitution occurs at residue 1187 of hJAGGED1.

Human bone marrow poly(A) RNA was obtained from Clontech Laboratories, Inc. (Palo Alto, CA) and reverse transcribed with random primer using the SuperScript Preamplication system (catalogue number 18089-011 from Gibco BRL (Gaithersburg, MD) following the manufacturer's procedure. First strand cDNA was subsequently amplified by PCR using degenerate primers SEQ ID NO:12 and SEQ ID NO:13, which correspond to peptide sequences DDFFGHY (residues 205-211; SEQ ID NO:16) and PCHYGGTCRDLVND (residues 676-689; SEQ ID NO:17), respectively. The sequence of SEQ ID NO:12 is 5'-GAYGAYTTYTTYGGNCAYTA-3', and the sequence of SEQ ID NO:13 is 5'-RCANGTNCCNCCRTARTGRCANGG-3', where R indicates G/C, Y indicates T/A, and N indicates G/C/T/A. PCR reactions were performed using Taq polymerase (Perkin Elmer, Foster City, CA) under the following conditions: 92°C, 30 seconds; 50°C, 30 seconds; and 72°C for 1 minute for 35 cycles. Ten candidate PCR products were obtained and cloned into the TA-cloning vector, pCR2l (Invitrogen, San Diego, CA). DNA sequencing was performed using the dyeprimer method with both M13 reverse and -21M13 primers on an ABI automated Sequencer model 377 or 373 (Applied Biosystems, Foster City, California). One of these clones was the 327 bp Sdi-06 clone described above.

To obtain the full-length hJAGGED1 cDNA, a human bone marrow λgt11 cDNA Library (catalogue number HL5005b; Clontech) was screened. The library was plated

at $5x10^4$ pfu on LB/Mg agar according to the manufacturer's protocol. After incubation for 8 to 12 hours, plaques were transferred to nitrocellulose filters (Schleicher & Schuell, Inc., Keene, NH) and denatured, neutralized, and cross-linked by UV irradiation. The filters were prehybridized and hybridized at 60°C with solutions prepared as described in Church and Kieffer-Higgins, Science 240:185-188 (1988), which is incorporated herein by reference. Following hybridization, filters were washed twice with 2XSSC/1%SDS for 10 minutes at room temperature and twice with 0.2XSSC/1%SDS for 20 minutes at 60°C. DNA was isolated from positive clones that were confirmed by a second hybridization under the same conditions. The cDNA clones D-01 and Y-A01, containing the 5' (2.2 kb) and 3' (4.5 kb) cDNA fragments of hJAGGED1, respectively, were cloned into the EcoRI site of the pBluescriptSK-vector (Stratagene, La Jolla, CA).

The full-length hJAGGED1 cDNA (pBS-hJg1) was generated by replacing the 300 bp 5' EcoRI/BglII fragment in Y-A01 with the 1.3 kb 5' EcoRI/BglII cDNA fragment in D-01. The resulting 5.5 kb cDNA clone hJAGGED1 was sequenced using random "shotgun" sequencing essentially as described in Smith et al., Genome Res. 6:1029-1049 (1996), which is incorporated herein by reference. A shotgun library was constructed by sonicating pBS-hJg1 plasmid DNA, size-selecting 1.5-2 kb fragments on an agarose gel, blunting the ends of the size-selected fragments using mung bean nuclease, and cloning the fragments into Sma I-digested M13-mp18 vector (Novagen, Inc., Madison, WI) essentially as described in Rowan and Koop (Eds.), Automated DNA Sequencing and Analysis pp. 167-174, Academic Press, Inc. (1994), and Smith et al., Genome Research 6:1029-1049 (1996), each of which is incorporated herein by reference. Briefly, single-stranded DNA was prepared from single plaques as described in Smith et al., *supra*, 1996. Approximately 80

single-stranded DNA templates were sequenced by ABI thermal-cycle sequencing using fluorescently-labeled -21M13 primer following the manufacturer's procedure. Sequencing data was assembled into a single 5.5 kb contig with approximately 6-fold redundancy using the basecalling and sequence assembly programs Phred and Phrap (P. Green, unpublished, http://www.genome, Washington.edu).

Expression of Human Jagged1 mRNA

In order to evaluate the expression pattern of hJAGGED1, Northern blot analysis was performed on multiple human tissues using a hJAGGED1 fragment as a probe. A single 5.5 kb mRNA transcript was detected in all tissues tested, including stomach, thyroid, spinal cord, lymph node, trachea, adrenal gland, and bone marrow. High levels of hJAGGED1 expression were noted in thyroid and trachea, while relatively lower levels of expression were observed in lymph node and bone marrow. Further Northern analysis demonstrated that hJAGGED1 is also expressed in adult heart, lung, skeletal muscle, kidney and placenta. However, hJAGGED1 expression was undetectable in adult brain or liver tissue.

Analysis of human fetal tissues showed high levels of hJAGGED1 expression in fetal kidney (16-32 weeks) and fetal lung (18-28 weeks), with lower levels of expression in fetal brain (20-25 weeks) and fetal liver (16-32 weeks). Expression of hJAGGED1 in heart, fetal liver, lung and kidney is consistent with a role for the hJAGGED1 protein in the normal development of these tissues.

The results described above demonstrate that hJAGGED1 is expressed in whole bone marrow, a heterogeneous tissue consisting of a variety of stromal

and hematopoietic cell populations. In order to determine whether hJAGGED1 expression is restricted to certain marrow subpopulations, RNA was isolated from primary human bone marrow stromal cells and analyzed by Northern blotting. A 5.5 kb transcript was detected, indicating that hJAGGED1 is expressed in bone marrow stromal cells. Several cell lines representing functionally distinct bone marrow stromal cells also were analyzed for hJAGGED1 expression. These immortalized human bone marrow stromal cell lines, designated HS-5, HS-23, and HS-27a, have been previously characterized (Roecklein and Torok-Storb, Blood 85:997-1005 (1995), which is incorporated herein by reference. The hJAGGED1 transcript was expressed at significant levels in HS-27a cells but was undetectable in HS-5 or HS-23 cells, indicating that hJAGGED1 is differentially expressed in distinct subpopulations of marrow stromal cells.

Northern blot analysis was performed as follows. Northern blots of multiple human tissues and human fetal tissues were obtained from Clontech and probed with ^{32}P-labeled Sdi-06 or a 400 bp fragment of the hJAGGED1 cDNA. The 400 bp probe was prepared by amplification with primer pair 292 (AGATCCTGTCCATGCAGAACGT; SEQ ID NO:18) and 293 (ATACTCAAAGTGGGCAACGCC; SEQ ID NO:19). For analysis of human stromal cells, 10 μg of total RNA was isolated from primary marrow stromal cells or the indicated stromal cell line using Stratagene's mRNA isolation kit (catalogue number 200347). Total RNA was electrophoresed on a formamide denaturing agarose gel and transferred onto Nytran® membrane (Schleicher & Schuell). Membranes were prehybridized and hybridized using Stratagene's QuikHyb® solution at 65°C. ^{32}P-labeled probes were denatured by boiling and added directly to prehybridization solution containing 100 μg salmon sperm DNA per 15 ml solution. Membranes were washed twice in

2X SSC/0.1% SDS at room temperature for 10 minutes, followed by washing once with 0.1X SSC/0.1% SDS at 60°C for 20 minutes. β-Actin cDNA (Clontech) was used as a control for the Northern analysis.

Expression of human JAGGED1 polypeptide

The full-length hJAGGED1 cDNA was cloned into the EcoRI/XhoI sites of the IPTG-inducible prokaryotic expression vector, pET-24b(+) (Novagen). The hJAGGED1 expression vector was transformed into B021(DE3) cells, which are bacterial cells containing the T7 RNA polymerase gene under control of an IPTG-inducible promoter.

A cell extract was prepared from transformed cells induced by 0.1 mM IPTG and from control uninduced cells. The cell extracts were fractionated on SDS-PAGE and transferred to nitrocellulose filters. Western analysis was performed with the ECL system (Amersham, Arlington Heights, IL) using a monoclonal antibody raised against peptide SEQ ID NO:11 ("J-C"), which corresponds to residues 1096 to 1114 of hJAGGED1 (KRRKPGSHTHSASEDNTTN). A polypeptide of about 150 kDa, absent from the control uninduced extract, was detected in the IPTG-induced cell extract. These results indicate that a hJAGGED1 polypeptide can be expressed in bacteria and that bacterially expressed hJAGGED1 exhibits a molecular weight of about 150 kDa.

EXAMPLE II

hJAGGED1 EXPRESSED ON MARROW STROMA INHIBITS HEMATOPOIETIC DIFFERENTIATION

This example demonstrates that a peptide derived from the DSL domain of hJAGGED1 inhibits G-CSF induced granulocytic differentiation of Notch1-expressing myeloid progenitors.

The HS-27a human stromal cell line inhibits differentiation of myeloid progenitors expressing Notch1

The ability of the hJAGGED1 HS-27a human stromal cell line to effect differentiation of hematopoietic progenitors was analyzed using the interleukin-3 (IL-3)-dependent myeloid cell line, 32D. The 32D cell line, which was derived from normal mouse bone marrow, is a heterogeneous cell line with individual cells having characteristics of myeloid cells at various early stages of maturation. 32D cells proliferate as undifferentiated blasts in the presence of IL-3, but differentiate into mature granulocytes when stimulated with granulocyte colony stimulating factor (G-CSF; Valtieri et al. Immunol. 138:3829-3835 (1987), which is incorporated herein by reference), thereby providing a system for analyzing factors that may affect myeloid differentiation.

Expression of an activated form of murine Notch1 inhibits G-CSF-induced granulocytic differentiation of 32D cells while permitting expansion of undifferentiated progenitor cells (Milner et al., *supra*, 1996). The function of hJAGGED1 was assayed by transducing 32D cells with a full-length Notch1 cDNA and evaluating the differentiative capacity of the transduced cells under several culture conditions. As shown in

Figure 3A, 32D clones expressing full-length Notch1 differentiate in response to G-CSF in a manner similar to parental 32D cells (WT) or clones expressing control retroviral constructs (LXSN). In contrast, 32D clones expressing the activated intracellular domain of Notch1 (N1-ICΔOP) remain primarily undifferentiated under these conditions, consistent with the results reported in Milner et al., *supra*, 1996 (Figure 3A).

Full-length Notch1-expressing 32D myeloid progenitors were co-cultured with hJAGGED1-expressing HS-27a human stromal cells, and differentiation of the 32D cells assayed. Figure 3B shows the differentiation patterns of 32D clones expressing full-length Notch1 or the control pLXSN retrovirus in the presence of G-CSF on monolayers of HS-27a, HS-23 or HS-5 stromal cells. LXSN control clones differentiate into mature granulocytes when cultured on any of these cell lines (Figure 3B, left panels); by day 6, 50-80% of the cells have a mature phenotype, and less than 15% remain undifferentiated. Full-length Notch1-expressing 32D cells also differentiate in response to G-CSF when cultured on the HS-23 or HS-5 lines, but granulocytic differentiation is significantly inhibited in the presence of HS-27a cells (Figure 3B, right panels). When cultured on HS-23 or HS-5 cells, 40-50% of the cells are mature with 15-20% remaining undifferentiated by day 6. In contrast, only 20% of the 32D cells are mature with 40% remaining undifferentiated when cultured on the HS-27a stromal cell line. The middle panels of Figure 3B show representative Wright stained cytospins of cells after four days in culture. The greatest difference between control and Notch1-expressing 32D cells occurs in the HS-27a co-cultures. These findings demonstrate that a specific interaction between HS-27a cells and Notch1 on 32D cells inhibits granulocytic differentiation, indicating that

hJAGGED1 is capable of activating Notch1 in myeloid progenitor cells.

The maintenance of undifferentiated progenitor cells was analyzed under different culture conditions by determining the total number of viable cells and the relative percentages of undifferentiated and mature cells remaining in the cultures on consecutive days. As shown in Table 1, cultures of 32D cells expressing full-length Notch1 maintain close to the original number of cells (90%) as undifferentiated progenitors after five days in G-CSF when cultured on HS-27a stromal cells. This result contrasts with cultures of control 32D cells, in which significantly fewer viable cells remain, almost all of which are differentiated. In the control 32D cells, fewer than 5% of the original number of cells are maintained as undifferentiated cells. Cultures of full-length Notch1-expressing 32D cells also had slightly greater numbers of undifferentiated cells remaining after five days when cultured on HS-23 or HS-5 stromal cells compared to cultures of the control 32D cells. However, cultures of full-length Notch1-expressing 32D cells grown on HS-27a contained significantly greater numbers of undifferentiated cells than those grown on either HS-23 or HS-5.

Table 1

Maintenance of undifferentiated cells after culture in the presence of G-CSF and stromal cell lines.

	Percent of original number of cells plated remaining undifferentiated			Replating efficiency
32D Clone	HS-27a	HS-23	HS-5	HS-27a
LXSN	5±4.7	4±3	2±1.2	11%
FL Notch1	90±28	15±2.6	19±29	190%

To verify that cells appearing undifferentiated by morphology were both viable and capable of continued proliferation as undifferentiated cells, cells were replated in WEH1 conditioned media (WCM) containing IL-3 after 6 days in culture with G-CSF and HS-27a cells. The cloning efficiency was evaluated by serial dilutions in 96-well plates as described further below. Compared to the original number of cells plated, the calculated percentage of clonable cells remaining was 190% for the full-length Notch1-expressing 32D cells and 11% for control 32D cells (see Table 1). These results indicate that co-culture of Notch1-expressing 32D cells in the presence of hJAGGED1-expressing HS-27a cells permits survival and maintains the proliferative potential of undifferentiated myeloid cells even in the presence of a differentiative stimulus such as G-CSF.

Notch1 cDNA retroviral vectors were constructed and transduced as follows. The full length clone of murine Notch1, provided by Drs. Jeff Nye and Raphael Kopan (Nye et al., Development 120:2421-2430 (1994); and Kopan and Weintraub, J. Cell Biol. 121:631-641 (1993), each of which is incorporated herein by reference) was subcloned into the EcoRI site of the pLXSN retroviral vector (Milner et al., *supra*, 1996). Retroviral producer cell lines expressing Notch1 were generated essentially as described in Milner et al., *supra*, 1996, and construct expression was confirmed by RT-PCR or western blot analysis. 32D cells were transduced by transwell co-cultivation with Notch1/PA317 producer cells as described in Milner et al., *supra*, 1996.

Notch1-expressing 32D clones were selected in G418 and expanded, and expression was confirmed by RT-PCR and western blotting using a monoclonal antibody generated against the intracellular domain of murine Notch1 provided by L. Milner.

The HS-27a, HS-23 and HS-5 human stromal cell lines were maintained in RPMI containing 10% FCS as described in Roecklein and Torok-Storb, *supra*, 1995. 32D cells were maintained in Iscove's Modified Dulbecco's Medium (IMDM) with 10% fetal bovine serum (FBS) and 10% WCM as a source of IL-3. For differentiation experiments, 32D cell lines were harvested in log phase, washed, counted, and replated at constant density ($2x10^5$ cells/ml, 4 ml/well) in 6-well plates in IMDM, 10% FBS, 0.5% WCM and 20 ng/ml recombinant human G-CSF from Amgen (Thousand Oaks, CA). Aliquots of 20 ml were removed daily for analysis and replaced with fresh media. Viable cells were counted, and Wright stained cytospins were evaluated for granulocytic differentiation as follows. Undifferentiated 32D cells generally had a single large, relatively round nucleus and scant dark blue cytoplasm containing few large granules. Criteria for granulocytic differentiation included nuclear segmentation, an increased cytoplasmic to nuclear ratio, and increased eosinophilia and granularity of the cytoplasm. Differential cell counts were performed on 100-200 cells on several occasions and in random/blinded fashion by the same individual (LM) to ensure consistency. The differential cell counts were confirmed by independent observers in a blinded fashion.

For co-culture experiments with 32D cells, human stromal cell lines were cultured in 6-well plates to approximately 75% confluence, washed and plated with 32D cells as described above, with the exception that 32D cells were plated at a density of $4x10^5$ cells/ml in 2 ml on the stromal cell layer and incubated for one to two hours prior to the addition of media containing G-CSF.

For assessment of cloning efficiency shown in Table 1, 32D cells were cultured at various cell densities ($2X10^5$, $1X10^4$, or $2.5X10^4$/ml) in 6-well plates

as described above. After 6 days in culture with media containing 20 ng/ml G-CSF and 0.5% WCM on HS-27a stromal layers, 32D cells were harvested and replated in triplicate in 10% WCM in 96-well plates. Serial dilutions were made, and wells assessed for growth daily for seven to ten days. Positive wells all showed continued proliferation during the period of observation and contained greater than 100 cells by day seven to ten.

A hJAGGED1 DSL peptide inhibits differentiation of Notch1-expressing myeloid progenitors

Three peptides corresponding to different regions of the hJAGGED1 molecule were analyzed for their effect on differentiation of Notch1-expressing 32D cells treated with G-CSF. Peptide SEQ ID NO:9 ("J-A") contains residues 188 to 204 of hJAGGED1 and corresponds to a hydrophilic portion of the conserved DSL domain, which is a domain unique to putative Notch ligands. Peptide SEQ ID NO:10 ("J-B") contains residues 235 to 257 and corresponds to part of EGF-repeat 1 in the extracellular domain. Peptide SEQ ID NO:11 ("J-C") contains residues 1096 to 1114 and corresponds to a hydrophilic portion of the intracellular domain. Figure 4 shows differentiation of control (LXSN) and full-length Notch1-expressing 32D cells in response to G-CSF in the presence of peptide SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:11. G-CSF-induced differentiation of control clones was unchanged by the addition of any of the peptides (Figure 4, left panels; compare to G-CSF alone in Figure 3A). Differentiation of the full-length Notch1-expressing 32D clones in the presence of G-CSF and either peptide SEQ ID NO:10 or SEQ ID NO:11 ("J-B" or "J-C"; Figure 4, top right) was comparable to that observed with G-CSF alone (see Figure 3A). In contrast, differentiation was significantly inhibited in the presence of peptide SEQ ID NO:9 ("J-A") (Figure 4, lower right). The extent of

inhibition was similar to that observed when these cells were co-cultured on the HS-27a monolayer in the presence of G-CSF (see Figure 3B).

Peptide SEQ ID NO:9 ("J-A") has the sequence CDDYYYGFGCNKFCRPR. Peptide SEQ ID NO:10 ("J-B") has the sequence CRQGCSPKHGSCKLPGDCRCQYG); and peptide SEQ ID NO:11 ("J-C") has the sequence KRRKPGSHTHSASEDNTTN. Each of these peptides were synthesized at the University of Washington Biopolymer Facility. Differentiation of 32D cells in the presence of hJAGGED peptides was analyzed as described above. 32D cells were incubated in media containing 20 μM peptide for 1 hour prior to the addition of G-CSF to a final concentration of 20 ng/ml. The final peptide concentration for the experiment depicted in Figure 4 was 10 μM. Fresh peptide was added to the original concentration on day 4 of culture.

An active fragment of hJAGGED1 inhibits granulocytic differentiation of mouse hematopoietic progenitor cells

A soluble fragment of hJAGGED1 (SEQ ID NO:7), which contains the extracellular domain of hJAGGED1 including the signal peptide, DSL region, EGF-like repeats and cysteine-rich region, was prepared by amplifying a portion of the hJAGGED1 cDNA with PCR primers 420 (SEQ ID NO:20; CCGCTCGAGACCATGCGTTCCCCACGGA) and 421 (SEQ ID NO:21; CGGAATTCTCAGTGGTGGTGGTGGTGGTGTTCATTGTTCGCTGAA). The hJAGGED1 cDNA fragment, corresponding to residues 1 to 1010, was subcloned into expression vector pDX to generate pDX-hJg1.Ex. After transfection into BHK and COS cells, the cell culture supernatant was assayed for the ability to effect the number of G-CFU formed from mouse hematopoietic progenitor cells (Sca-1$^+$ lin$^-$), which were prepared by removing cells that stained with anti-Gr-1, anti-CD4, anti-CD11b, anti-CD2, anti-CD45R and

anti-Ter-119 and then positively selecting Sca-1$^+$ cells with anti-Sca-1. As shown in Table 2, supernatant from BHK cells transfected with the hJAGGED1 extracellular domain construct reduced the average number of colony forming units (CFU-G-CSF) of Sca-1$^+$ lin$^-$ cells treated with G-CSF from about 60 to about 24. These results indicate that the hJAGGED1 fragment SEQ ID NO:7 encoding the extracellular domain of hJAGGED1 (residues 1 to 1010) inhibits granulocytic differentiation and is an active fragment of hJAGGED1.

Table 2

Number of CFU-G-CSF		
Sample	Supernatant of BHK cells	Supernatant of BHK cells transfected with pDX-hJg1.Ex
Sample 1	99	34
Sample 2	48	20
Sample 3	45	23
Sample 4	48	19
Average	60	24

A cDNA fragment corresponding to the DSL region of hJAGGED1 (amino acids 178 to 240; SEQ ID NO:8) was amplified using primer 517 (SEQ ID NO:22; CGCGGATCCTCAGCCTTGTCGGCAAATAGC) and 518 (SEQ ID NO:23; CCCAAGCTTGCCCACTTTGAGTATCAGA). The fragment was subcloned into the PinPoint™ expression vector (Promega, Madison, WI), and expressed as a fusion protein with a peptide that becomes biotinylated in *E. coli*. After purification of the hJAGGED1 DSL fragment using avidin chromatography, the biotin-tagged hJAGGED1 fragment was assayed for activity in a high proliferative potential (HPP) assay with sorted mouse hematopoietic stem cells

(Sca-1$^+$, lin$^-$) as described in Patel et al., J. Exp. Med. 185:1163-1172 (1997), which is incorporated herein by reference). The HPP assay is an assay to test the self-renewal capacity of hematopoietic progenitor cells. Sorted mouse hematopoietic progenitor cells (Sca$^+$, lin$^-$) were cultured with a combination of growth factors (IL-1, IL-3 and stem cell factor) with or without 50-100 nM biotin-tagged hJAGGED1 DSL fragment SEQ ID NO:8 on soft agar for 10 days. The results of this assay demonstrated that the hJAGGED1 fragment SEQ ID NO:8 increased HPP efficiency two-fold. Thus, the hJAGGED1 fragment SEQ ID NO:8, corresponding to residues 178 to 240 of hJAGGED1, is an active fragment of JAGGED that increases the self-renewal capacity of hematopoietic progenitor cells.

EXAMPLE III

MAPPING hJAGGED1 RELATIVE TO THE ALAGILLE SYNDROME CRITICAL REGION

This example describes the mapping of the human JAGGED1 gene to chromosome 20p12.

hJAGGED1 Maps to Chromosome 20p12

In order to obtain a probe for fluorescence *in situ* hybridization (FISH), a total genomic library from Research Genetics (Huntsville, AL) was screened with the hJAGGED1 cDNA fragment Sdi-06. Two genomic bacterial artificial chromosome (BAC) clones, 49-D9 and 125-B1, were isolated, and the presence of the hJAGGED1 gene demonstrated by Southern blot analysis.

Probes were ^{32}P-labeled with PrimIt-II following the manufacturer's procedure (Stratagene, La Jolla, CA). Fluorescence *in situ* hybridization was performed with

each BAC clone independently. Both 49-D9 and 125-B1 hybridized specifically to 20p12 in a metaphase spread. FISH signals were observed at 20p12 on both chromosomes in each of the 10 metaphase cells analyzed and were not consistently observed at any other location. These results indicate that the hJAGGED1 gene maps to chromosome 20p12.

Fluorescence *in situ* hybridization was performed essentially as described in Trask, "Fluorescence in situ hybridization" in Birren et al., (Eds.) Genome Analysis: A Laboratory Manual Cold Spring Harbor Laboratory Press (1997) and Krantz, Am. J. Med Genet. 70:80-86 (1997), each of which is incorporated herein by reference. Briefly, BAC DNA was biotinylated by nick translation and hybridized to metaphase preparations (2 ng probe/μl). Human Cotl DNA (GIBCO-BRL) was added to the hybridization solution at a final concentration of 100 ng/ml to prevent hybridization of labeled repetitive sequences to chromosome spreads. Metaphase preparations were obtained from phytohemagglutinin-stimulated peripheral blood lymphocyte cultures that were blocked in early S-phase with methotrexate and released to (pro)metaphase in the presence of bromodeoxyuridine. Hybridization sites were detected with avidin-FITC, and chromosomes were banded with DAPI at 2 μg/ml in an antifade solution. FITC and DAPI images were collected separately, but in registration, using Spectrum Analytics IPLab Spectrum 3.0 software, a Princeton CCD camera (KAF 1400 chip), a Ludl filter-wheel equipped with ChromaTechnology excitation filters, and a Zeiss AxioPhot microscope equipped with a 100x, 13 N.A. objective and a ChromaTechnology multi-band pass emission filter. The images were pseudocolored and merged after the DAPI-banding contrast was enhanced by applying a 5x5 linear HAT filter supplied with the IPLab package. More than 10 metaphases were analyzed from the

computer screen or by direct visualization through the microscope.

Mapping hJAGGED1 relative to the Alagille Syndrome critical region.

Studies of the minimal region of overlap of multiple patients with cytogenetic deletions have defined an Alagille Syndrome critical region at chromosome 20p12 between genetic markers D20S41 and D20S162 (Figure 5). A contig of YAC, P1 and BAC clones spanning the critical region was used to further define this region. The distal boundary of the region is defined by a P1 clone (20p1-158), containing the synaptosomal associated protein-25 (SNAP-25). This clone was present in two copies in the patient with the most centromeric deletion (Krantz et al., *supra*, 1997). The centromeric boundary of the region is defined by P-1243b12, which is outside of the deletion in the patient with the most distal deletion. The size of this critical region is estimated at 1.2 to 1.3 Mb. Two BAC clones 49D9 and 125B1, which contain part of the hJAGGED1 gene, map to the 20p12 region. Using multiple PCR primers 249/250 (SEQ ID NOS: 24 and 25) and 247/248 (SEQ ID NOS:26 and 27) from BAC clone 49D9, on a panel of YAC, P1 and BAC clones, hJAGGED1 was sublocalized between D20S894 and D20S507 within the Alagille Syndrome critical region (see Figure 5).

CEPH human YAC clones were identified through the Whitehead Institute for Biomedical Research/MIT Center for Genomic Research web site and published data (Pollet et al., Genomics 27:467-474 (1995), which is incorporated herein by reference) and provided by Dr. Marcia Budarf (CHOP). The human P1 Library (Shepherd et al., Proc. Natl. Acad. Sci. 91:2629-2633 (1994), which is incorporated herein by reference) was screened

essentially as described in Stokke et al., Genomics 26:134-7 (1995), which is incorporated herein by reference. The human BAC library Stokke et al., *supra*, 1995; Shizuya et al., Proc. Natl. Acad. Sci. 89:8794-8797 (1992), which is incorporated herein by reference) was screened according to the protocol supplied by Research Genetics. Selected clones were mapped by FISH and STS content analysis to confirm cytogenetic localization and to order the clones. When clones were not contiguous, clone ends were obtained by sequencing using T7 and SP6 promoters, and new PCR primers were designed based on the sequence for the next round of library screening. Sequencing was carried out in the Nucleic Acid Sequencing Cores at the University of Pennsylvania, Department of Genetics, and at The Children's Hospital of Philadelphia. Fluorescence in situ hybridization studies were carried out by standard techniques essentially as described in, Krantz et al., *supra*, 1997, which is incorporated herein by reference.

Microsatellite markers were amplified as follows. $(TTTG)_n$ was amplified with primer pair 249/250 (GGTCTTTTGCCACTGTTT; SEQ ID NO:24 and GAATAGGGAGGAGAAAAC; SEQ ID NO:25), and $(GTTT)_n$ was amplified with primer pair 247/248 (GTCTTTTGCCACTGTTTG; SEQ ID NO:26 and GAATAGGGAGGAGAAAAC; SEQ ID NO:27).

EXAMPLE IV

hJAGGED1 GENE STRUCTURE

This example describes the identification of the hJAGGED exon/intron boundaries.

Identification of hJAGGED1 exon/intron boundaries

DNA array technology was used to determine the exon/intron boundaries of the hJAGGED1 gene as described in Nguyen et al., Genomics 29:207-216 (1995), which is incorporated herein by reference. BAC clone 49D9 was fragmented by sonication, and fragments ranging in size from 1.5 to 2 kb were selected and ligated into an M13 bacteriophage vector. Individual single stranded M13 clones were picked into 384-well microfilter plates, and 1,536 clones were arrayed onto four sets of nylon membranes using a 384-pin Replicator. The arrays of the BAC 49D9 M13 fragments were hybridized with the full length hJAGGED1 cDNA. All positive M13 clones (approximately 100 clones) were picked and sequenced. The hJAGGED1 genomic and cDNA sequences were aligned, and 47 intron/exon boundaries were defined (Figure 6A and 6B). The sequences from the 5' end, upstream of base pair 803 of the hJAGGED1 cDNA sequence, were missing one or two exons, presumably because the 5' end of the gene is not contained in the BAC 49D9 clone (Figure 6A). The 5' identified exons are indicated exon (n+1), where n stands for the unknown number of missing exons (see Figure 6B). The intron/exon and exon/intron boundary sequences of hJAGGED1 exons 3 through 26 are shown in Figure 6B as SEQ ID NOS:28 through 74.

BAC DNA sequence analysis was performed using random shot-gun sequencing essentially as described

above. Approximately 100 single-strand DNA templates were cloned into pCR.2.1 vector using the TA cloning system from Invitrogen. DNA was prepared using 5'-3' DNA mini-preparation system (5'prime-3'prime, Inc., Boulder, CO) and sequenced. Fluorescently-labeled -21M13 primer was used for sequencing of single-stranded DNA, and fluorescently labeled -21M13 and M13 forward primers were used for sequencing of double-stranded cDNA following the manufacture's procedure (ABI).

EXAMPLE V

ALAGILLE SYNDROME ASSOCIATED hJAGGED1 MUTATIONS

This example describes the association of several independent hJAGGED1 coding sequence mutations with Alagille Syndrome in four Alagille families.

Heteroduplex Mobility Analysis (HMA) of Alagille Syndrome Families

The hJAGGED1 gene contains at least 26 exons, and its mRNA is 5.5 kb in length. Heteroduplex mobility analysis (HMA) was used to screen for Alagille Syndrome-associated mutations in six RT-PCR products spanning the hJAGGED1 mRNA. HMA analysis is an assay that can readily detect mutations in heterozygotes at a given locus and is therefore potentially useful in screening for mutations in dominant disorders (Delwart et al., Science 262:1257-1261 (1993), which is incorporated herein by reference). Initially, ten individuals from four Alagille Syndrome families, each with multiple affected members, were screened by HMA (Figure 7). None of these families demonstrated deletions of 20p12 by cytogenetic or molecular analyses. RT-PCR was performed with six primer pairs to generate small overlapping cDNA fragments, designated A, B, C, D, E and F, which span

most of the hJAGGED1 coding sequence (Figure 7A). After localizing the mutation within one of the six amplified fragments, the cDNA region was sequenced and the identity of the mutation confirmed at the genomic level as described further below.

Shown in Figure 9 are the normal CNRAICRQGCS (SEQ ID NO:103) and corresponding mutant CNSYLPTRLQS* (SEQ ID NO:104) amino acid sequences of Alagille Syndrome family 1; the normal WCGPRPCL (SEQ ID NO:105) and corresponding mutant WCGVALDL (SEQ ID NO:106) amino acid sequences of Alagille Syndrome family 2; the normal DSQCD (SEQ ID NO:107) and corresponding mutant DSVMR (SEQ ID NO:108) amino acid sequences of Alagille Syndrome family 3; and the normal FCKCPED (SEQ ID NO:109) and corresponding mutant FCKCPRT (SEQ ID NO:110) amino acid sequences of Alagille Syndrome family 4.

Analysis of Alagille Syndrome Family 1

HMA analysis of family 1 indicated a mobility shift in PCR product "B" in two affected individuals (Figure 7B). Sequence analysis of the hJAGGED1 cDNAs from affected family members demonstrated a deletion of nucleotides "AG" at positions 1104 and 1105. To confirm that the two nucleotide deletion in the "B" region causes the mobility shift detected by HMA, cloned RT-PCR products from affected and unaffected family members were analyzed. cDNA with the "AG" deletion in combination with clones from a non-deleted individual produced an expected mobility shift identical to that of cDNAs from the RT-PCR products (Figure 7F and 7B). As anticipated, HMA analysis of each individual clone did not lead to the mobility shift. Fifteen cDNA clones from the "B" region were sequenced from each individual analyzed. Normal sequences were detected in all individuals in this family, but affected individuals demonstrated both mutant

and normal alleles. The "AG" deletion lies in exon (n+2).

Single strand conformational polymorphism (SSCP) analysis of exon 4 (designated exon n+2) on the extended family revealed a mobility shift in the three affected individuals in this family (Figure 8A). Furthermore, this deletion was confirmed by sequence analysis of the genomic DNA of exon (n+2) (Figure 8A). The disease-associated and normal nucleotide sequences of Alagille Syndrome family 1 in the region of this deletion are shown in Figure 8A as SEQ ID NOS:75 and 76, respectively. The "AG" deletion leads to a reading frame shift at residue 230, positioned at the end of the DSL domain, and is predicted to result in premature termination at residue 240. Thus, the "AG" deletion in family 1 results in a truncated hJAGGED1 protein lacking the 979 C-terminal residues (see Figure 9).

The two affected brothers in this family have liver disease, heart disease including pulmonic and peripheral pulmonic stenosis, posterior embryotoxon and Alagille facies. Their less severely affected mother has a heart murmur, posterior embryotoxon and Alagille facies.

Analysis of Alagille Syndrome Family 2

HMA analysis was similarly performed on family 2. PCR products from two affected members of family 2 showed mobility shifts in the "D" region (Figure 7C). cDNA sequence analysis of amplified "D" region sequences from both affected individuals revealed two changes: a five nucleotide insertion (GTGGC) at position 3102 and an 86 nucleotide deletion from nucleotides 2785 to 2871. The insertion is a repeat of the GTGGC sequence at positions 3102-3107. The 86 nucleotide deletion was

seen in all three members of this family, one of whom is unaffected, and corresponds to a complete absence of exon 23 (exon n+21). This result indicates that this exon can be removed from the final transcript by alternative splicing and that the 86 nucleotide deletion does not correlate with disease phenotype. Analyses in the "D" region of 10 individuals from four families identified a common heteroduplex. This observation is consistent with the presence of transcripts both containing and deleting exon (n+18) in all individuals tested (Figure 7B, C and D).

The multiple bands seen by HMA in the "D" region corresponded to the three types of variation identified by sequencing: a 5 bp insertion, a 86 bp deletion, and both a 5 bp insertion and an 86 bp deletion. Three cloned cDNA fragments, generated by PCR using the "D" region primers from individuals in Alagille Syndrome family 2, were tested. Each clone contains one variant. A clone from AGS2-2 (AGS $2\text{-}2_1$) contained the 5 nucleotide insertion. A clone from AGS 2-3 contained the 86 nucleotide deletion, and a third clone from AGS2-2 ($AGS2\text{-}2_2$) contained the 5 nucleotide insertion in addition to the 86 nucleotide deletion. These clones were hybridized with the normal clone D-n1 and analyzed by HMA. As shown in Figure 7E, these three types of hybridizations correspond to the heteroduplexes seen. These results indicate that only the five bp insertion correlates with the Alagille Syndrome disease phenotype. This disease-associated 5 bp insertion was localized to exon (n+21).

SSCP analysis revealed a novel band in this exon, present in an affected father and daughter and absent in the unaffected mother and in 50 normal control individuals (Figure 7C). The disease-associated and normal nucleotide sequences of Alagille Syndrome family 2

in the region of the mutation are shown in Figure 8B as SEQ ID NOS:77 and 78, respectively. The insertion, which was confirmed by genomic sequence analysis of the mutant hJAGGED1 genes in both affected individuals, is predicted to result in a translational frameshift downstream of codon 898. Translation is predicted to terminate at codon 945, resulting in a truncated hJAGGED1 protein lacking the C-terminal 274 residues. The mutant protein is predicted to contain the DSL domain, the entire EGF repeat domain, and about a third of the cysteine-rich domain, with an additional segment of 47 residues altered by the translational frameshift. The remainder of the cysteine-rich domain, the transmembrane (TM) domain and the intracellular region have been deleted (see Figure 9).

The phenotypes of the two affected individuals in this family are different. The father has liver disease, cardiac disease, and renal failure, while his daughter is more mildly affected with characteristic facies and pulmonary artery stenosis but normal liver and kidney function to date.

Analysis of Alagille Syndrome Family 3

The two affected individuals in this family showed shifts in the "C" region PCR products (Figure 7D). Sequence analysis revealed a four nucleotide "CAGT" deletion at positions 2531-2534 in exon (n+15) in both affected individuals. HMA analysis of a cDNA clone carrying the "CAGT" deletion, and a clone from a normal family member demonstrated a mobility shift (Figure 7F) identical to the RT-PCR products (Figure 7D).

SSCP analysis of exon (n+15) revealed a novel band in the affected proband, her affected mother, and in the DNA from the conceptus of a terminated pregnancy

(Figure 8C). The SSCP variant was not identified in 50 control individuals (100 chromosomes). The four nucleotide deletion was confirmed by genomic sequencing of exon 17 (exon n+15) from the affected individuals (Figure 8C). The disease-associated and normal nucleotide sequences of Alagille Syndrome family 3 in the region of the deletion are shown in Figure 8C as SEQ ID NOS:79 and 80, respectively. The mutant gene is predicted to encode an hJAGGED1 protein having a translational frameshift at residue 741 with an altered segment of 33 amino acids before chain termination. The translational frameshift occurs in the 12th EGF repeat as shown in Figure 9.

The proband in this family was severely affected, with liver involvement, severe branch pulmonary artery stenosis, butterfly vertebrae, and posterior embyrotoxon. She died at 2.5 years of age from head trauma after a fall. Her mother has a milder phenotype coming to medical attention at 20 years of age during pre-surgical evaluation for a basilar artery aneurysm. Studies at that time revealed abnormal liver function; further tests revealed bile duct paucity, pulmonic stenosis, characteristic facies and posterior embryotoxon with retinal changes.

Analysis of Alagille Syndrome Family 4

No heteroduplexes were seen in any of the six PCR products from individuals in this family (Figure 7D and 7F). However, cDNA sequence analysis revealed a single "C" nucleotide deletion at position 2066 in an affected daughter and father (Figure 7D). This deletion lies in exon (n+11).

SSCP analysis of exon (n+11) revealed an altered band in the proband and her father (Figure 8D).

Genomic sequence analyses verified the presence of the "C" deletion in exon 13 (exon n+11) in both affected family members. The disease-associated and normal nucleotide sequences of Alagille Syndrome family 4 in the region of the nucleotide deletion are shown in Figure 8D as SEQ ID NOS:81 and 82, respectively. The deletion shown in Figure 8D is predicted to result in a translational frameshift at residue 550 followed by an altered 13 residue segment before chain termination in EGF repeat 9 (Figure 9).

The proband was severely affected with liver and heart disease (tetralogy of Fallot), facial features of Alagille Syndrome, butterfly vertebrae and posterior embryotoxon. She died at 5 years of age from sepsis. Her father was mildly affected with a history of a heart murmur and characteristic facies. Liver studies were normal; an ophthalmology exam has yet to be conducted. The proband's sibling is also apparently affected, having severe congenital heart disease (tetralogy of Fallot) and posterior embyrotoxon. Her liver studies have been normal.

The Alagille Syndrome patients studied were subject to a complete diagnostic examination. All probands met the diagnostic criteria for the disorder. The proband of each family had Alagille syndrome as judged by the presence of bile duct paucity in addition to a minimum of three of the five following clinical criteria: cholestasis, cardiac disease, vertebral anomalies, anterior chamber defects of the eye and characteristic facial features. Additional family members were examined or their medical records reviewed. All patients and their families were enrolled in the study under an IRB approved protocol at the Children's Hospital of Philadelphia.

RT-PCR and Heteroduplex Mobility Analysis was performed as follows. Total RNA was isolated using Trizol RNA isolation kit (GIBCO-BRL), and cDNA was synthesized using GIBCO/BRL's reverse transcription system following the manufacture's procedure. Taq polymerase (Perkin Elmer) was used to amplify one-twentieth the volume of the reverse transcribed cDNA. The hJAGGED1 cDNA "A" segment was amplified with primers 292/395 (AGATCCTGTCCATGCAGAACGT; SEQ ID NO:83 and CATCCAGCCTTCCATGCAA; SEQ ID NO:84); the "B" segment was amplified with primers 398/399 (CTTTGAGTATCAGATCCGCGTGA; SEQ ID NO:85 and CGATGTCCAGCTGACAGA; SEQ ID NO:86); the "C" segment was amplified with primers 402/403 (CGGGATTTGGTTAATGGTTAT; SEQ ID NO:87 and GGTACCAGTTGTCTCCAT; SEQ ID NO:88); the "D" segment was amplified with primers 406/407 (GGAACAACCTGTAACATAGC; SEQ ID NO:89 and GGCCACATGTATTTCATTGTT; SEQ ID NO:90; the "E" segment was amplified with primers 408/409 (GAATATTCAATCTACATCGCTT; SEQ ID NO:91 and CTCAGACTCGAGTATGACACGA; SEQ ID NO:92); and the "F" segment was amplified with primers 410/411 (AAAGTGCCCAGAGCTTAAACCG; SEQ ID NO:93 and GGTGTTTTAAACATCTGACGTCGTA; SEQ ID NO:94).

Heteroduplex mobility analysis was performed using the following procedure: 200-500 ng of DNA was denatured at 96°C for five minutes in denaturing buffer (0.1M NaCl, 10 mM Tris HCl (pH 7.8), and 2 mM EDTA). The denatured DNA was immediately removed to a wet ice bath for five minutes and subsequently incubated at 55°C for five minutes. The reannealed DNA was mixed with loading buffer (0.2% Orange G, 2.5% Ficoll) and electrophoresed on a 5% polyacrylamide gel (19.5 X 19 cm) in 1X TBE buffer for 3 to 3.5 hours at 250 volts. After

electrophoresis, the gel was stained in 0.5 μg/ml ethidium bromide.

SSCP analysis was performed as follows. DNA was extracted from lymphocytes (whole blood) or established lymphoblastoid cell lines of affected and unaffected members of each Alagille family and from unrelated normal control subjects using the Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN). The primers for PCR analysis were designed to cover all exons as well as the intron/exon boundaries of hJAGGED1 as outlined in Figure 6B. For SSCP analysis, each PCR reaction contained 75 ng of genomic DNA, 200 μM dATP, dTTP, and dGTP, and 62.5 μM dCTP, 4 μCi of ^{32}P-dCTP, 10 pM of each primer, 1.0-1.5 mM $MgCl_2$, 2.5 μl dimethyl sulfoxide, 2.5 μl of 10X PCR Buffer II (Perkin Elmer, Foster City, CA), and 0.75 U AmpliTaq polymerase (Perkin Elmer) in a final volume of 25 μl. Exon (n+4) was amplified with primer pair 510/511 (CAGGGAAGAAGGCTGCAATGT; SEQ ID NO:95 and TGGTGGGGTGATAAATGGACAC; SEQ ID NO:96); exon (n+11) was amplified with primer pair 447/448 (GTTTTACTCTGATCCCTC; SEQ ID NO:97 and CAAGGGGCAGTGGTAGTAAGT; SEQ ID NO:98); exon (n+15) was amplified with primer pair 455/456 (GCTATCTCTGGGACCCTT; SEQ ID NO:99 and CCACGTGGGGCATAAAGTT; SEQ ID NO:100); and exon (n+21) was amplified with primer pair 467/468 (ATGGCTGCCGCAGTTCA; SEQ ID NO:101 and CAAGCAGACATCCACCAT; SEQ ID NO:102). PCR conditions were as follows: 94°C, 30 seconds; 50°C, 1 minute; and 72°C, 30 seconds for 35 cycles.

The denatured PCR products were analyzed by electrophoresis on MDE gels (FMC Corp., Pinebrook, NJ) with and without glycerol at 4°C for 4-5 hours. Gels were transferred to filter paper and exposed to X-ray film at 70°C for 1 to 24 hours. Amplicons demonstrating SSCP band shifts were sequenced by the Nucleic

Acid/Protein core facility of the Children's Hospital of Philadelphia using an ABI373A automated sequencer.

All journal article, reference, and patent citations provided above, in parentheses or otherwise, whether previously stated or not, are incorporated herein by reference.

Although the invention has been described with reference to the disclosed embodiments, those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention. It should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:

1. An isolated JAGGED peptide having at most about 40 amino acids, comprising substantially the same amino acid sequence as SEQ ID NO:9.

2. The isolated JAGGED peptide of claim 1, comprising the amino acid sequence SEQ ID NO:9.

3. The isolated JAGGED peptide of claim 2, consisting of the amino acid sequence SEQ ID NO:9.

4. A method of inhibiting differentiation of hematopoietic progenitor cells, comprising contacting said progenitor cells with an isolated JAGGED polypeptide comprising substantially the same amino acid sequence as JAGGED, or an active fragment thereof.

5. The method of claim 4, wherein said progenitor cells are contacted *in vitro*.

6. The method of claim 4, wherein said isolated JAGGED polypeptide comprises substantially the same amino acid sequence as SEQ ID NO:2 or SEQ ID NO:4, or an active fragment thereof.

7. The method of claim 6, wherein said active fragment is a soluble fragment.

8. The method of claim 7, wherein said soluble fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NO:7 and SEQ ID NO:8.

9. A method of inhibiting differentiation of progenitor cells, comprising contacting said progenitor cells with an isolated JAGGED peptide having at most about 40 amino acids and comprising substantially the same amino acid sequence as SEQ ID NO:9.

10. The method of claim 9, wherein said cells are hematopoietic progenitor cells.

11. The method of claim 9, wherein said cells are contacted *in vitro*.

12. The method of claim 9, wherein said isolated JAGGED peptide comprises the amino acid sequence SEQ ID NO:9.

13. The method of claim 12, wherein said isolated JAGGED peptide consists of the amino acid sequence SEQ ID NO:9.

14. A method of maintaining progenitor cells in an undifferentiated state, comprising contacting said progenitor cells with a JAGGED polypeptide, or active fragment thereof.

15. The method of claim 14, wherein said progenitor cells are capable of reconstituting the hematopoietic system.

16. The method of claim 14, wherein said progenitor cells are maintained in a totipotent state.

17. The method of claim 16, wherein said progenitor cells are maintained in a totipotent state in culture.

18. The method of claim 14, further comprising cryopreservation of said progenitor cells maintained in an undifferentiated state.

19. The method of claim 14, further comprising introducing a nucleic acid molecule encoding a gene product into said progenitor cells.

20. A method of maintaining progenitor cells in an undifferentiated state, comprising contacting said progenitor cells with a JAGGED peptide having at most about 40 amino acids and comprising substantially the same amino acid sequence as SEQ ID NO:9.

21. The method of claim 20, wherein said progenitor cells are capable of reconstituting the hematopoietic system.

22. The method of claim 20, wherein said progenitor cells are maintained in a totipotent state.

23. The method of claim 22, wherein said progenitor cells are maintained in a totipotent state in culture.

24. The method of claim 20, further comprising cryopreservation of said progenitor cells maintained in an undifferentiated state.

25. The method of claim 20, further comprising introducing a nucleic acid molecule encoding a gene product into said progenitor cells.

26. A method of diagnosing Alagille Syndrome in an individual, comprising detecting an Alagille Syndrome disease-associated mutation linked to a JAGGED locus.

27. The method of claim 26, wherein said disease-associated mutation is within a JAGGED gene.

28. The method of claim 27, wherein said disease-associated mutation is within a JAGGED coding sequence.

29. The method of claim 26, wherein said JAGGED locus is a human JAGGED1 (hJAGGED1) locus.

30. The method of claim 29, wherein said disease-associated mutation produces a truncated hJAGGED1 gene product.

31. The method of claim 30, wherein said disease-associated mutation occurs within the hJAGGED1 nucleotide sequence SEQ ID NO:1 at a position selected from group consisting of nucleotides 1104-1105, nucleotide 3102, nucleotides 2531-2534 and nucleotide 2066.

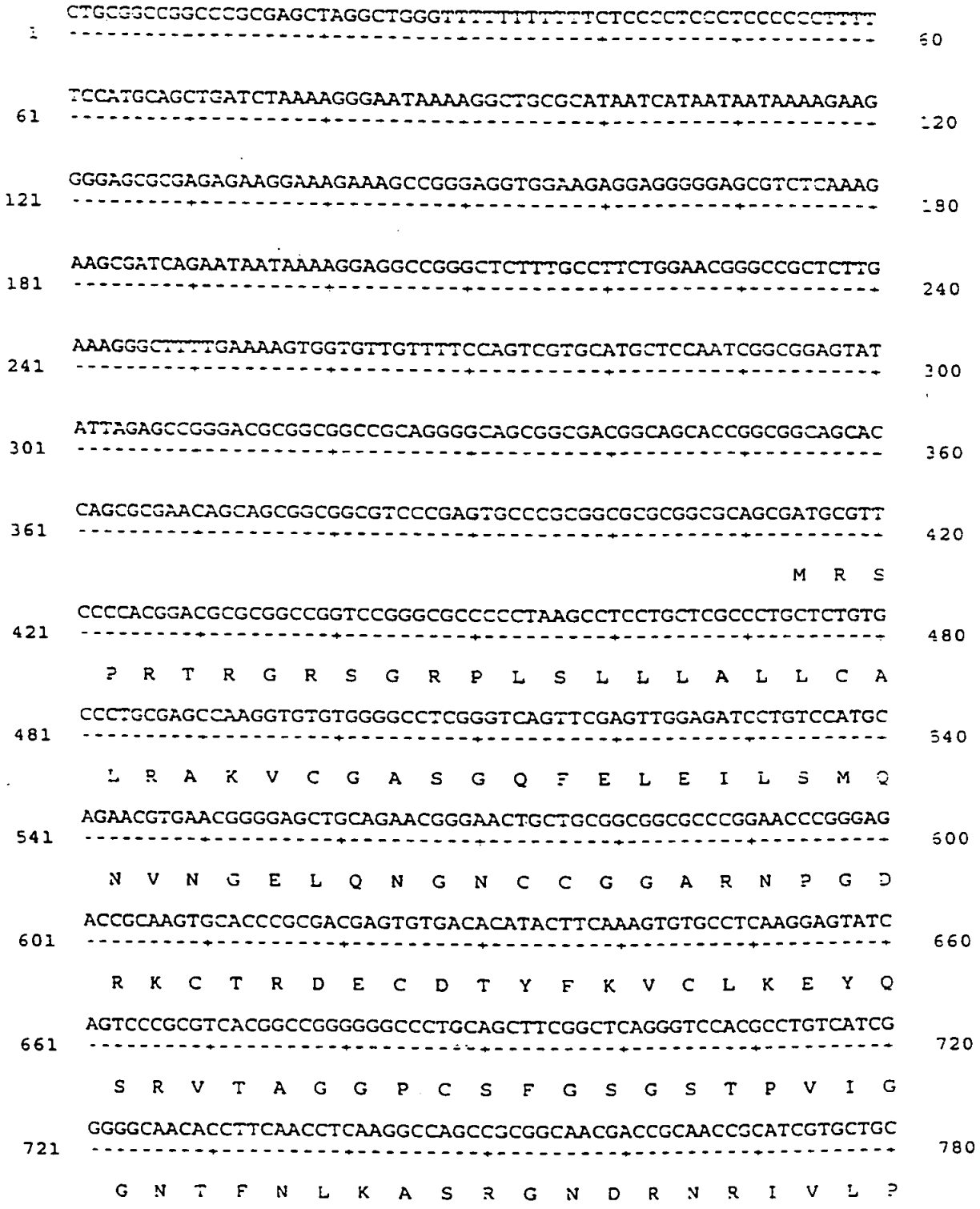

Figure 1A
(Page 1 of 8)

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                CTTTCAGTTTCGCCTGGCCGAGGTCCTATACGTTGCTTGTGGAGGCGTGGGATTCCAGTA
781             ----------+---------+---------+---------+---------+---------- 840
                  F  S  F  A  W  P  R  S  Y  T  L  L  V  E  A  W  D  S  S  N

                ATGACACCGTTCAACCTGACAGTATTATTGAAAAGGCTTCTCACTCGGGCATGATCAACC
841             ----------+---------+---------+---------+---------+---------- 900
                  D  T  V  Q  P  D  S  I  I  E  K  A  S  H  S  G  M  I  N  P

                CCAGCCGGCAGTGGCAGACGCTGAAGCAGAACACGGGCGTTGCCCACTTTGAGTATCAGA
901             ----------+---------+---------+---------+---------+---------- 960
                  S  R  Q  W  Q  T  L  K  Q  N  T  G  V  A  H  F  E  Y  Q  I

                TCCGCGTGACCTGTGATGACTACTACTATGGCTTTGGCTGCAATAAGTTCTGCCGCCCCA
961             ----------+---------+---------+---------+---------+---------- 1020
                  R  V  T  C  D  D  Y  Y  Y  G  F  G  C  N  K  F  C  R  P  R

                GAGATGACTTCTTTGGACACTATGCCTGTGACCAGAATGGCAACAAAACTTGCATGGAAG
1021            ----------+---------+---------+---------+---------+---------- 1080
                  D  D  F  F  G  H  Y  A  C  D  Q  N  G  N  K  T  C  M  E  G

                GCTGGATGGGCCCCGAATGTAACAGAGCTATTTGCCGACAAGGCTGCAGTCCTAAGCATG
1081            ----------+---------+---------+---------+---------+---------- 1140
                  W  M  G  P  E  C  N  R  A  I  C  R  Q  G  C  S  P  K  H  G

                GGTCTTGCAAACTCCCAGGTGACTGCAGGTGCCAGTATGGCTGGCAAGGCCTGTACTGTG
1141            ----------+---------+---------+---------+---------+---------- 1200
                  S  C  K  L  P  G  D  C  R  C  Q  Y  G  W  Q  G  L  Y  C  D

                ATAAGTGCATCCCACACCCGGGATGCGTCCACGGCATCTGTAATGAGCCCTGGCAGTGCC
1201            ----------+---------+---------+---------+---------+---------- 1260
                  K  C  I  P  H  P  G  C  V  H  G  I  C  N  E  P  W  Q  C  L

                TCTGTGAGACCAACTGGGGCGGCCAGCTCTGTGACAAAGATCTCAATTACTGTGGGACTC
1261            ----------+---------+---------+---------+---------+---------- 1320
                  C  E  T  N  W  G  G  Q  L  C  D  K  D  L  N  Y  C  G  T  H

                ATCAGCCGTGTCTCAACGGGGGAACTTGTAGCAACACAGGCCCTGACAAATATCAGTGTT
1321            ----------+---------+---------+---------+---------+---------- 1380
                  Q  P  C  L  N  G  G  T  C  S  N  T  G  P  D  K  Y  Q  C  S

                CCTGCCCTGAGGGGTATTCAGGACCCAACTGTGAAATTGCTGAGCACGCCTGCCTCTCTG
1381            ----------+---------+---------+---------+---------+---------- 1440
                  C  P  E  G  Y  S  G  P  N  C  E  I  A  E  H  A  C  L  S  D

                ATCCCTGTCACAACAGAGGCAGCTGTAAGGAGACCTCCCTGGGCTTTGAGTGTGAGTGTT
1441            ----------+---------+---------+---------+---------+---------- 1500
                  P  C  H  N  R  G  S  C  K  E  T  S  L  G  F  E  C  E  C  S
```

Figure 1A

(Page 2 of 8)

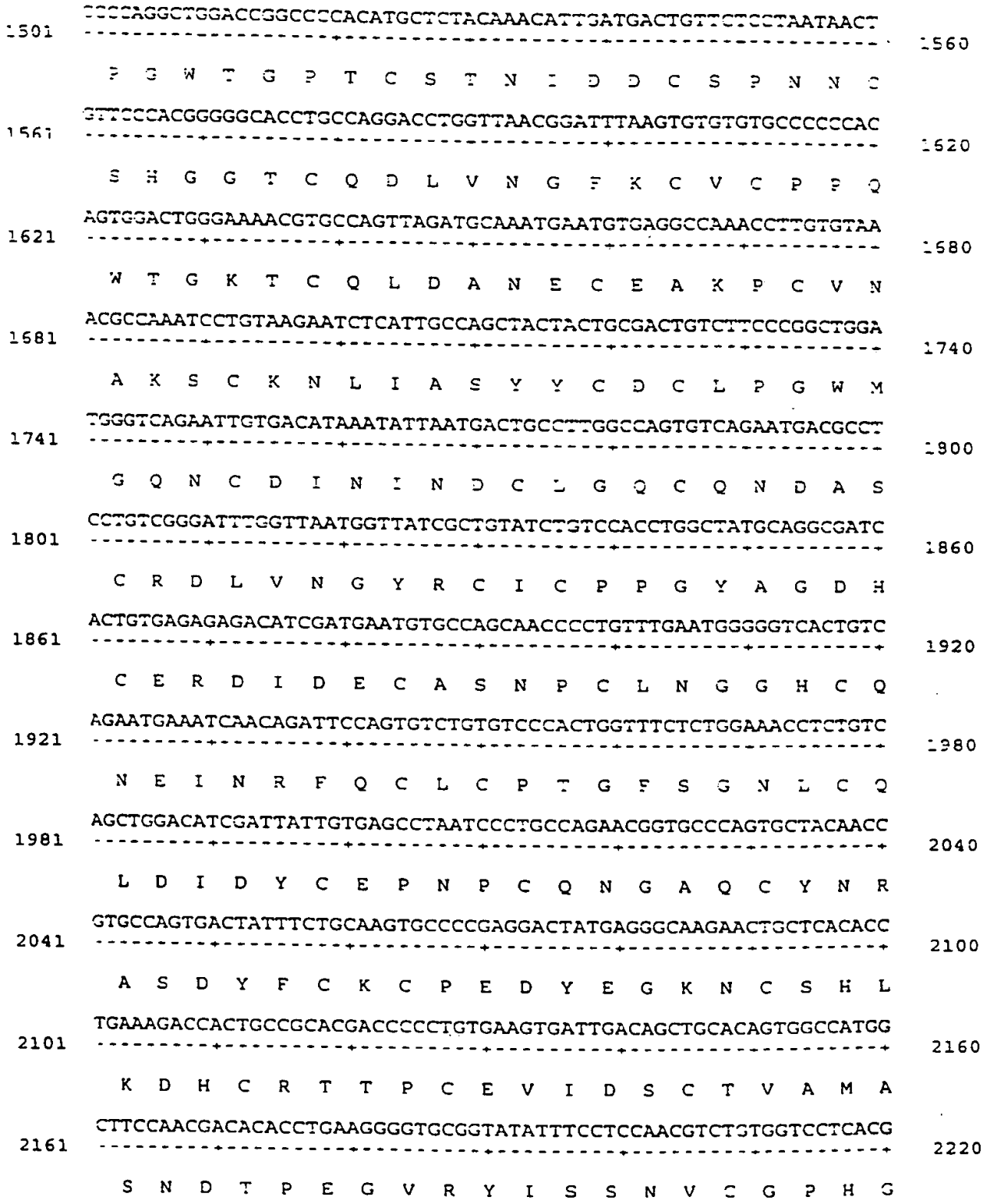


Figure 1A

(Page 3 of 8)

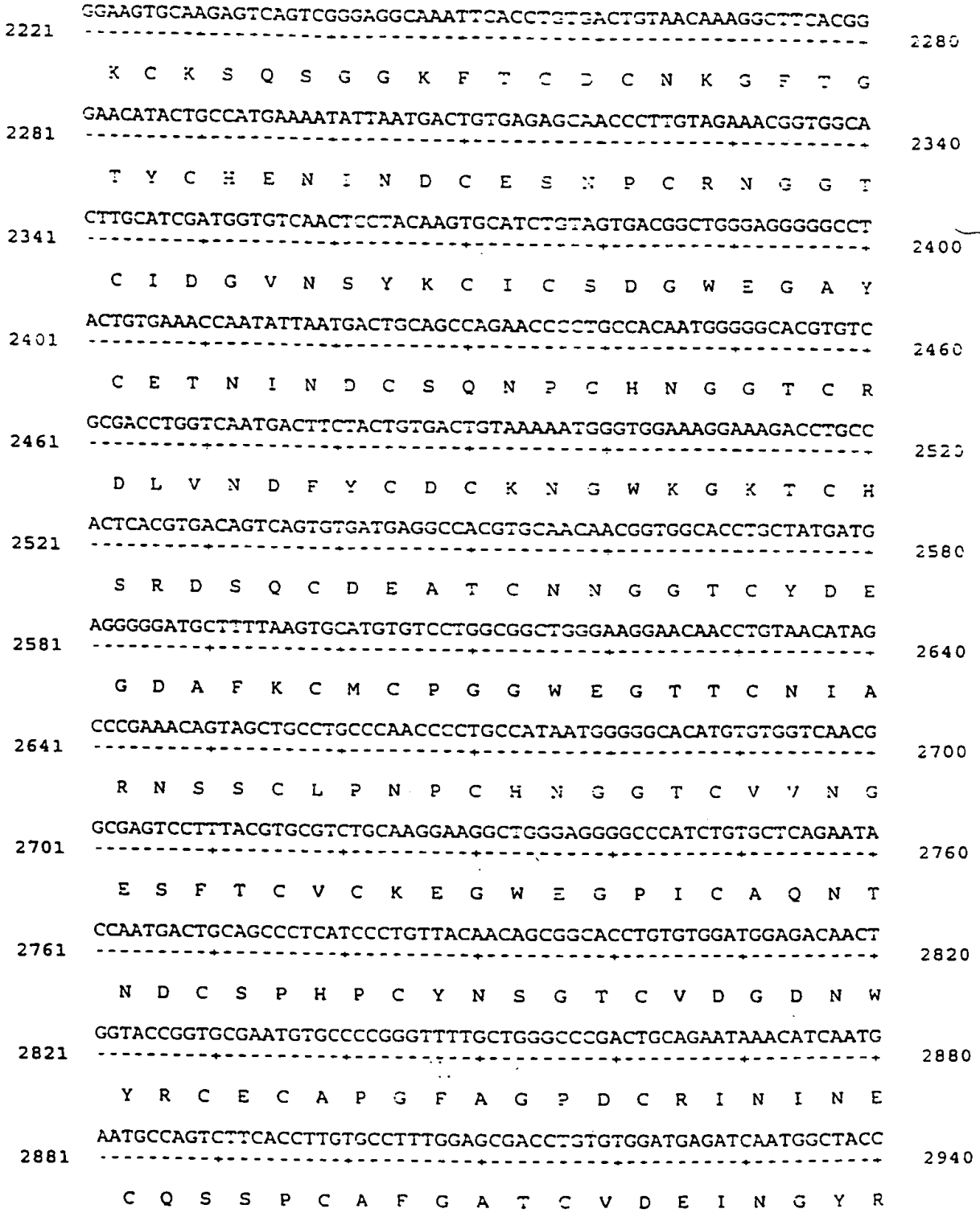


Figure 1A

(Page 4 of 8)

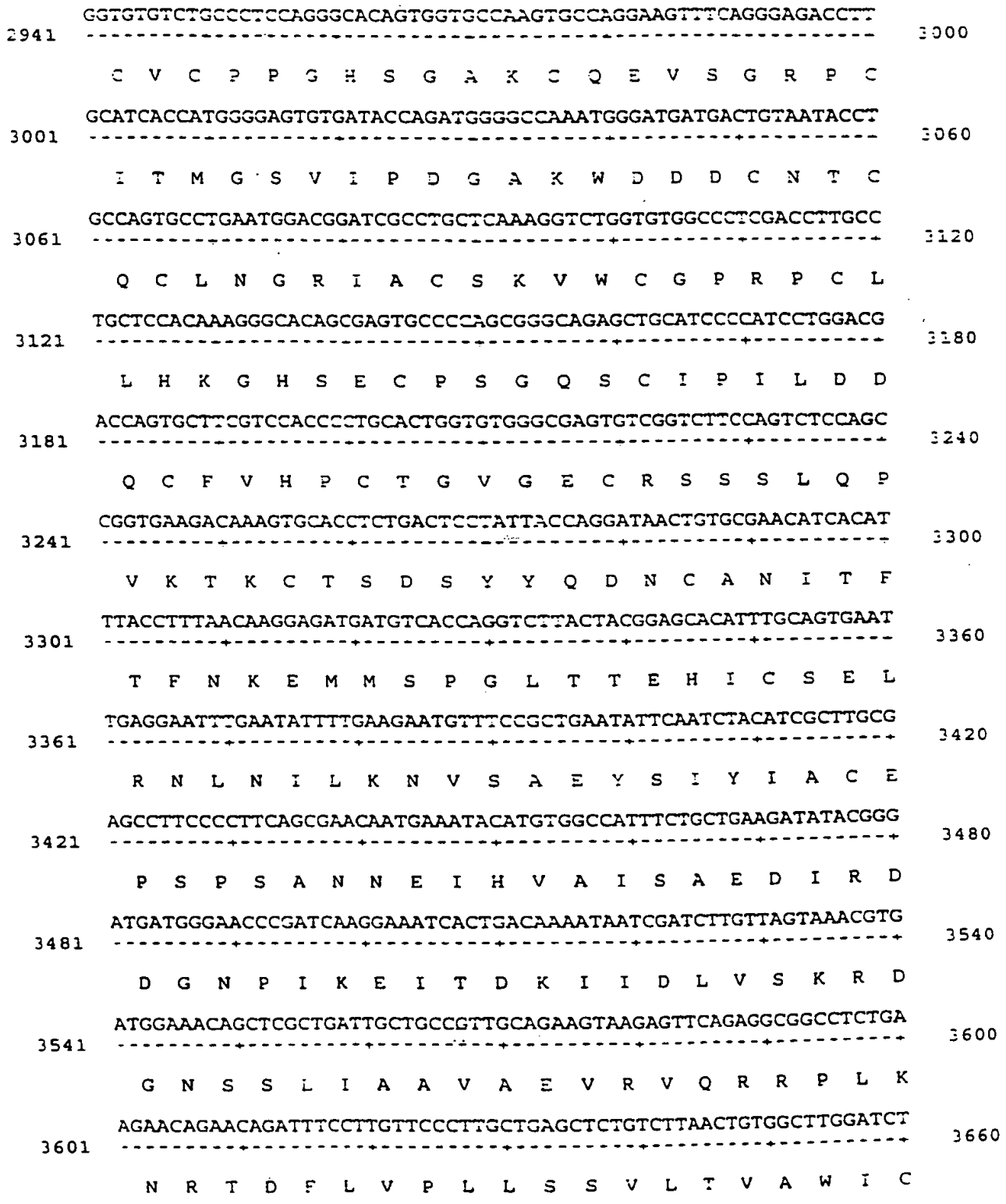


Figure 1A
(Page 5 of 8)

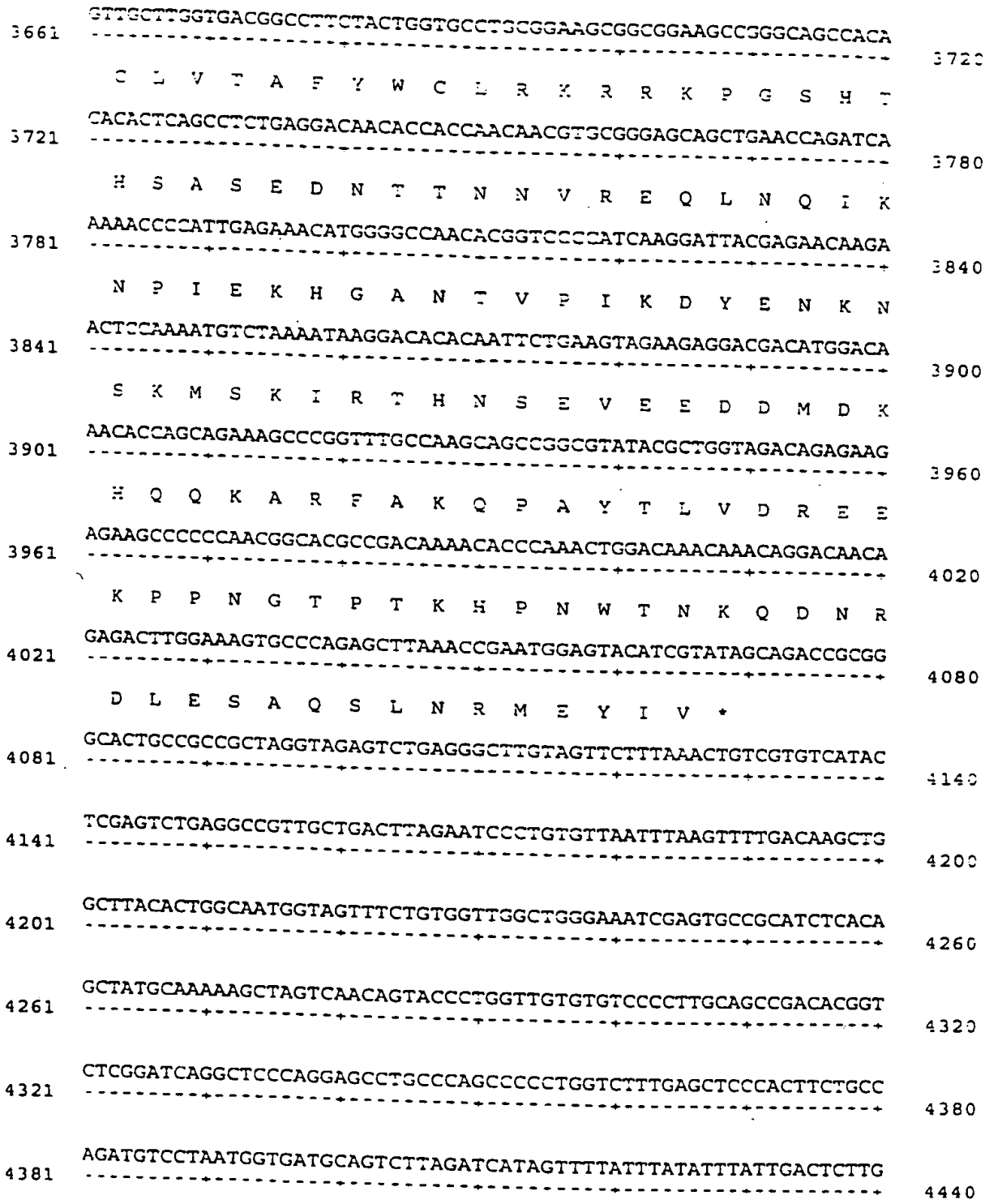


Figure 1A
(Page 6 of 8)

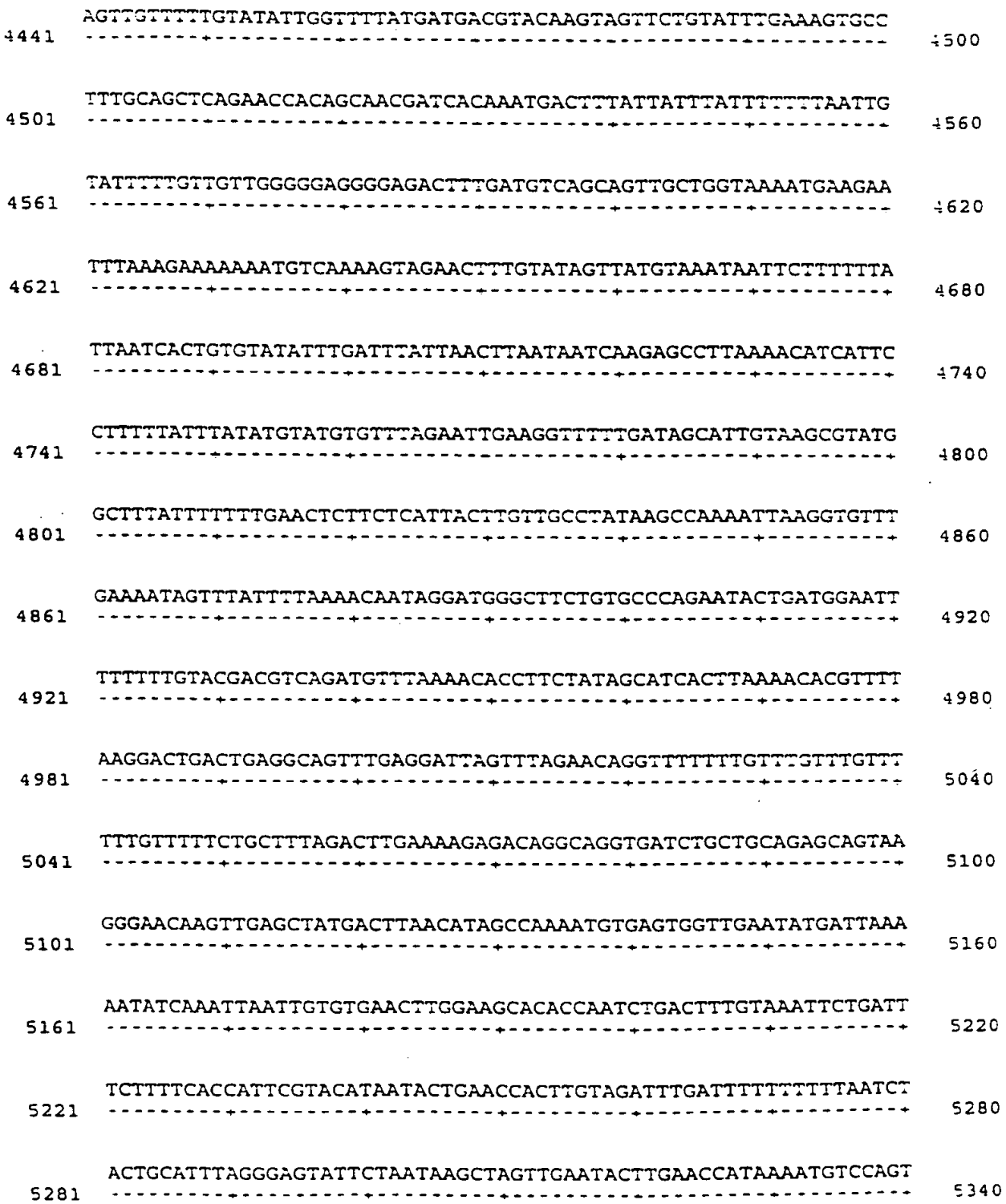


Figure 1A
(Page 7 of 8)

```
5341  AAGATCACTGTTTAGATTTGCCATAGAGTACACTGCCTGCCTTAAGTGAGGAAATCAAAG
      ---------+---------+---------+---------+---------+---------+ 5400

5401  TGCTATTACGAAGTTCAAGATCAAAAAGGCTTATAAAACAGAGTAATCTTGTTGGTTCAC
      ---------+---------+---------+---------+---------+---------+ 5460

5461  CATTGAGACCGTGAAGATACTTTGTATTGTCCTATTAGTGTTATATGAACATACAAATGC
      ---------+---------+---------+---------+---------+---------+ 5520

5521  ATCTTTGATGTGTTGTTCTTGGCAATAAATTTTGAAAAGTAATATTTATTAAATTTTTTT
      ---------+---------+---------+---------+---------+---------+ 5580

5581  GTATGAAAAC
      ----------+ 5590
```

Figure 1A
(Page 8 of 8)

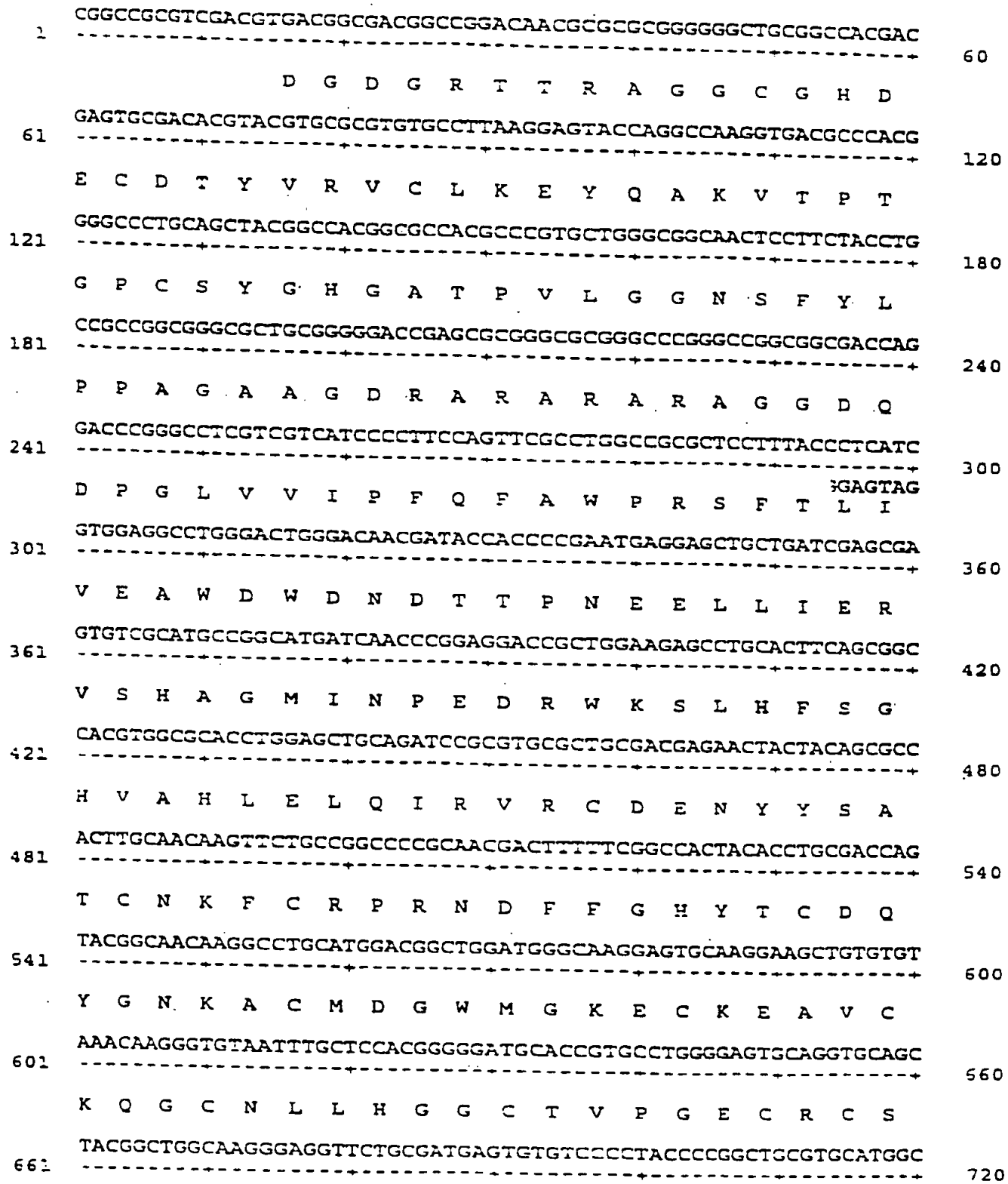


Figure 1B
Page 1 of 6

```
     Y  G  W  Q  G  R  F  C  D  E  C  V  P  Y  P  G  C  V  H  G

     AGTTGTGTGGAGCCCTGGCAGTGCAACTGTGAGACCAACTGGGGCGGCCTGCTCTGTGAC
721  ---------+---------+---------+---------+---------+---------+  780

     S  C  V  E  P  W  Q  C  N  C  E  T  N  W  G  G  L  L  C  D

     AAAGACCTGAACTACTGTGGCAGCCACCACCCCTGCACCAACGGAGGCACGTGCATCAAC
781  ---------+---------+---------+---------+---------+---------+  840

     K  D  L  N  Y  C  G  S  H  H  P  C  T  N  G  G  T  C  I  N

     GCCGAGCCTGACCAGTACCGCTGCACCTGCCCTGACGGCTACTCGGGCAGGAACTGTGAG
841  ---------+---------+---------+---------+---------+---------+  900

     A  E  P  D  Q  Y  R  C  T  C  P  D  G  Y  S  G  R  N  C  E

     AAGGCTGAGCACGCCTGCACCTCCAACCCGTGTGCCAACGGGGGCTCTTGCCATGAGGTG
901  ---------+---------+---------+---------+---------+---------+  960

     K  A  E  H  A  C  T  S  N  P  C  A  N  G  G  S  C  H  E  V

     CCGTCCGGCTTCGAATGCCACTGCCCATCGGGCTGGAGCGGGCCCACCTGTGCCCTTGAC
961  ---------+---------+---------+---------+---------+---------+ 1020

     P  S  G  F  E  C  H  C  P  S  G  W  S  G  P  T  C  A  L  D

     ATCGATGAGTGTGCTTCGAACCCGTGTGCGGCCGGTGGCACCTGTGTGGACCAGGTGGAC
1021 ---------+---------+---------+---------+---------+---------+ 1080

     I  D  E  C  A  S  N  P  C  A  A  G  G  T  C  V  D  Q  V  D

     GGCTTTGAGTGCATCTGCCCCGAGCAGTGGGTGGGGGCCACCTGCCAGCTGGACGTCAAC
1081 ---------+---------+---------+---------+---------+---------+ 1140

     G  F  E  C  I  C  P  E  Q  W  V  G  A  T  C  Q  L  D  V  N

     GACTGTCGCGGGCAGTGTCAGCATGGGGGCACCTGCAAGGACCTGGTGAACGGGTACCAG
1141 ---------+---------+---------+---------+---------+---------+ 1200

     D  C  R  G  Q  C  Q  H  G  G  T  C  K  D  L  V  N  G  Y  Q

     TGTGTGTGCCCACGGGGCTTCGGAGGCCGGCATTGCGAGCTGGAACGAGACAAGTGTGCC
1201 ---------+---------+---------+---------+---------+---------+ 1260

     C  V  C  P  R  G  F  G  G  R  H  C  E  L  E  R  D  K  C  A

     AGCAGCCCCTGCCACAGCGGCGGCCTCTGCGAGGACCTGGCCGACGGCTTCCACTGCCAC
1261 ---------+---------+---------+---------+---------+---------+ 1320

     S  S  P  C  H  S  G  G  L  C  E  D  L  A  D  G  F  H  C  H

     TGCCCCCAGGGCTTCTCCGGGCCTCTCTGTGAGGTGGATGTCGACCTTTGTGAGCCAAGC
1321 ---------+---------+---------+---------+---------+---------+ 1380

     C  P  Q  G  F  S  G  P  L  C  E  V  D  V  D  L  C  E  P  S

     CCCTGCCGGAACGGCGCTCGCTGCTATAACCTGGAGGGTGACTATTACTGCGCCTGCCCT
1381 ---------+---------+---------+---------+---------+---------+ 1440
```

Figure 1B
Page 2 of 6

```
      P  C  R  N  G  A  R  C  Y  N  L  E  G  D  Y  Y  C  A  C  P
      GATGACTTTGGTGGCAAGAACTGCTCCGTGCCCCGCGAGCCGTGCTGGCGGGGCCTGCAG
1441  ----------+---------+---------+---------+---------+---------+  1500

      D  D  F  G  G  K  N  C  S  V  P  R  E  P  C  W  R  G  L  Q
      AGTGATCGATGGCTGCGGGTCAGACGCGGGGCCTGGGATGCCTGGCACAGCACGTCCGGC
1501  ----------+---------+---------+---------+---------+---------+  1560

      S  D  R  W  L  R  V  R  R  G  A  W  D  A  W  H  S  T  S  G
      GTGTGTGGCCCCCATGGACGCTGCGTCAGCCAGCCAGGGGGCAACTTTTCCTGCATCTGT
1561  ----------+---------+---------+---------+---------+---------+  1620

      V  C  G  P  H  G  R  C  V  S  Q  P  G  G  N  F  S  C  I  C
      GACAGTGGCTTTACTGGCACCTACTGCCATGAGAACATTGACGACTGCCTGGGCCAGCCC
1621  ----------+---------+---------+---------+---------+---------+  1680

      D  S  G  F  T  G  T  Y  C  H  E  N  I  D  D  C  L  G  Q  P
      TGCCGCAATGGGGGCACATGCATCGATGAGGTGGACGCCTTCCGCTGCTTCTGCCCCAGC
1681  ----------+---------+---------+---------+---------+---------+  1740

      C  R  N  G  G  T  C  I  D  E  V  D  A  F  R  C  F  C  P  S
      GGCTGGGAGGGCGAGCTCTGCGACACCAATCCCAACGACTGCCTTCCCGATCCCTGCCAC
1741  ----------+---------+---------+---------+---------+---------+  1800

      G  W  E  G  E  L  C  D  T  N  P  N  D  C  L  P  D  P  C  H
      AGCCGCGGCCGCTGCTACGACCTGGTCAATGACTTCTACTGTGCGTGCGACGACGGCTGG
1801  ----------+---------+---------+---------+---------+---------+  1860

      S  R  G  R  C  Y  D  L  V  N  D  F  Y  C  A  C  D  D  G  W
      AAGGGCAAGACCTGCCACTCACGCGAGTTCCAGTGCGATGCCTACACCTGCAGCAACGGT
1861  ----------+---------+---------+---------+---------+---------+  1920

      K  G  K  T  C  H  S  R  E  F  Q  C  D  A  Y  T  C  S  N  G
      GGCACCTGCTACGACAGCGGCGACACCTTCCGCTGCGCCTGCCCCCCCGGCTGGAAGGGC
1921  ----------+---------+---------+---------+---------+---------+  1980

      G  T  C  Y  D  S  G  D  T  F  R  C  A  C  P  P  G  W  K  G
      AGCACCTGCGCCGTCGCCAAGAACAGCAGCTGCCTGCCCAACCCCTGTGTGAATGGTGGC
1981  ----------+---------+---------+---------+---------+---------+  2040

      S  T  C  A  V  A  K  N  S  S  C  L  P  N  P  C  V  N  G  G
      ACCTGCGTGGGCAGCGGGGCCTCCTTCTCCTGCATCTGCCGGGACGGCTGGGAGGGTCGT
2041  ----------+---------+---------+---------+---------+---------+  2100

      T  C  V  G  S  G  A  S  F  S  C  I  C  R  D  G  W  E  G  R
      ACTTGCACTCACAATACCAACGACTGCAACCCTCTGCCTTGCTACAATGGTGGCATCTGT
2101  ----------+---------+---------+---------+---------+---------+  2160
```

Figure 1B
Page 3 of 6

```
      T  C  T  H  N  T  N  D  C  N  P  L  P  C  Y  N  G  G  I  C
      GTTGACGGCGTCAACTGGTTCCGCTGCGAGTGTGCACCTGGCTTCGCGGGGCCTGACTGC
2161  ---------+---------+---------+---------+---------+---------  2220
      V  D  G  V  N  W  F  R  C  E  C  A  P  G  F  A  G  P  D  C
      CGCATCAACATCGACGAGTGCCAGTCCTCGCCCTGTGCCTACGGGGCCACGTGTGTGGAT
2221  ---------+---------+---------+---------+---------+---------  2280
      R  I  N  I  D  E  C  Q  S  S  P  C  A  Y  G  A  T  C  V  D
      GAGATCAACGGGTATCGCTGTAGCTGCCCACCCGGCCGAGCCGGCCCCCGGTGCCAGGAA
2281  ---------+---------+---------+---------+---------+---------  2340
      E  I  N  G  Y  R  C  S  C  P  P  G  R  A  G  P  R  C  Q  E
      GTGATCGGGTTCGGGAGATCCTGCTGGTCCCGGGGCACTCCGTTCCCACACGGAAGCTCC
2341  ---------+---------+---------+---------+---------+---------  2400
      V  I  G  F  G  R  S  C  W  S  R  G  T  P  P  P  H  G  S  S
      TGGGTGGAAGACTGCAACAGCTGCCGCTGCCTGGATGGCCGCCGTGACTGCAGCAAGGTG
2401  ---------+---------+---------+---------+---------+---------  2460
      W  V  E  D  C  N  S  C  R  C  L  D  G  R  R  D  C  S  K  V
      TGGTGCGGATGGAAGCCTTGTCTGCTGGCCGGCCAGCCCGAGGCCCTGAGCGCCCAGTGC
2461  ---------+---------+---------+---------+---------+---------  2520
      W  C  G  W  K  P  C  L  L  A  G  Q  P  E  A  L  S  A  Q  C
      CCACTGGGGCAAAGGTGCCTGGAGAAGGCCCCAGGCCAGTGTCTGCGACCACCCTGTGAG
2521  ---------+---------+---------+---------+---------+---------  2580
      P  L  G  Q  R  C  L  E  K  A  P  G  Q  C  L  R  P  P  C  E
      GCCTGGGGGGAGTGCGGCGCAGAAGAGCCACCGAGCACCCCCTGCCTGCCACGCTCCGGC
2581  ---------+---------+---------+---------+---------+---------  2640
      A  W  G  E  C  G  A  E  E  P  P  S  T  P  C  L  P  R  S  G
      CACCTGGACAATAACTGTGCCCGCCTCACCTTGCATTTCAACCGTGACCACGTGCCCCAG
2641  ---------+---------+---------+---------+---------+---------  2700
      H  L  D  N  N  C  A  R  L  T  L  H  F  N  R  D  H  V  P  Q
      GGCACCACGGTGGGCGCCATTTGCTCCGGGATCCGCTCCCTGCCAGCCACAAGGGCTGTG
2701  ---------+---------+---------+---------+---------+---------  2760
      G  T  T  V  G  A  I  C  S  G  I  R  S  L  P  A  T  R  A  V
      GCACGGGACCGCCTGCTGGTGTTGCTTTGCGACCGGGCGTCCTCGGGGGCCAGTGCCGTG
2761  ---------+---------+---------+---------+---------+---------  2820
      A  R  D  R  L  L  V  L  L  C  D  R  A  S  S  G  A  S  A  V
      GAGGTGGCCGTGTCCTTCAGCCCTGCCAGGGACCTGCCTGACAGCAGCCTGATCCAGGGC
2821  ---------+---------+---------+---------+---------+---------  2880
```

Figure 1B
Page 4 of 6

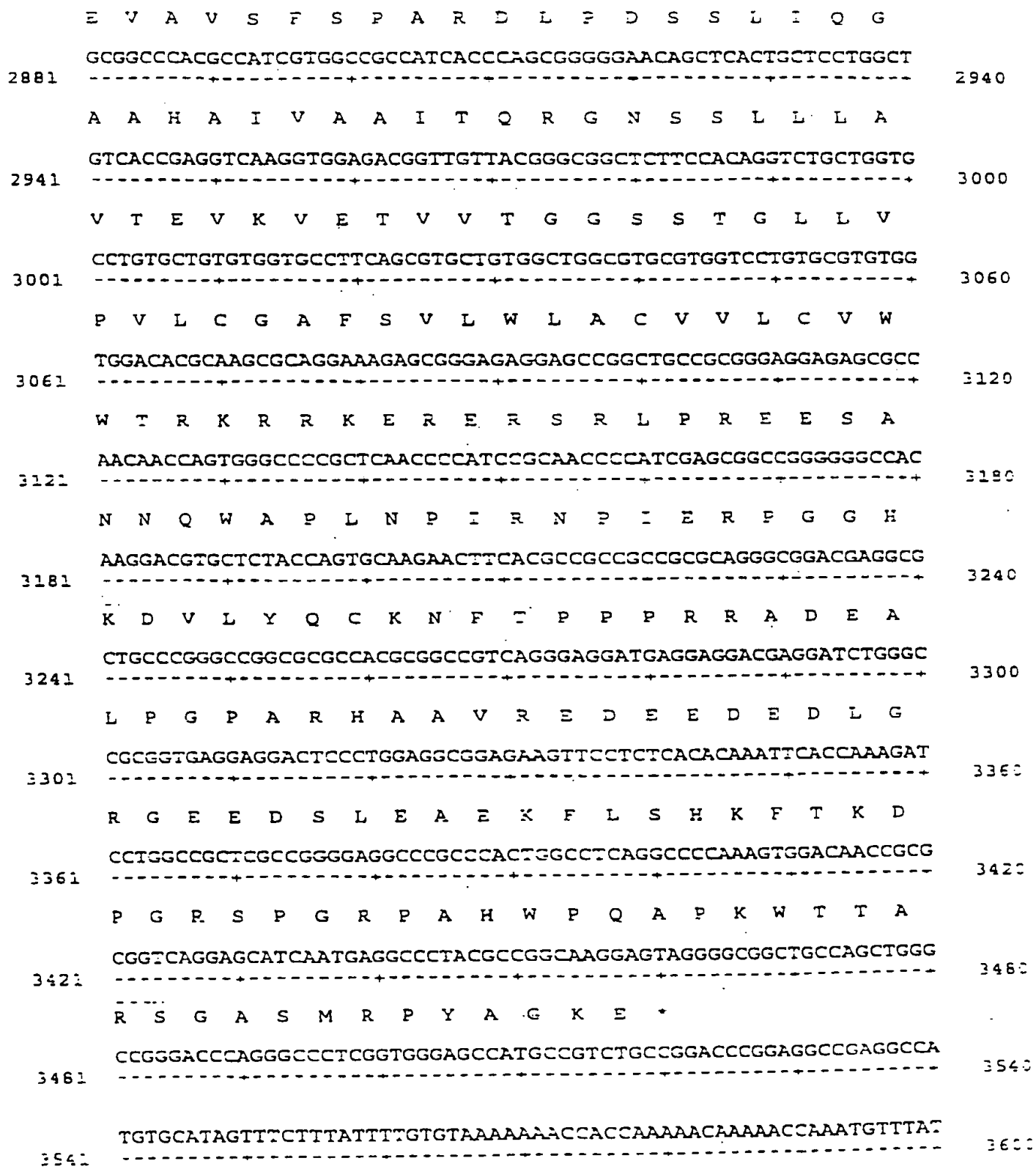


Figure 1B
Page 5 of 6

```
3601 TTTCTACGTTTCTTTAACCTTGTATAAATTATTCAGTAACTGTCAGGCTGAAAACAATGG 3660

3661 AGTATTCTCGGATAGTTGCTATTTTTGTAAAGTTTCCGTGCGTGGCACTCGCTGTATGAA 3720

3721 AGGAGAGAGCAAAGGGTGTCTGCGTCGTCACCAAATCGTAGCGTTTGTTACCAGAGGTTG 3780

3781 TGCACTGTTTACAGAATCTTCCTTTTATTCCTCACTCGGGTTTCTCTGTGGCTCCAGGCC 3840

3841 AAAGTGCCGGTGAGACCCATGGCTGTGTTGGTGTGGCCCATGGCTGTTGGTGGGACCCGT 3900

3901 GGCTGATGGTGTGGCCTGTGGCTGTCGGTGGGACTCGTGGCTGTCAATGGGACCTGTGGC 3960

3961 TGTCGGTGGGACCTACGGTGGTCGGTGGGACCCTGGTTATTGATGTGGCCCTGGCTGCCG 4020

4021 GCACGGCCCGTGGCTGTTGACGCACCTGTGGTTGTTAGTGGGGCCTGAGGTCATCGGCGT 4080

4081 GGCCCAAGGCCGGCAGGTCAACCTCGCGCTTGCTGGCCAGTCCACCCTGCCTGCCGTCTG 4140

4141 TGCTTCCTCCTGCCCAGAACGCCCGCTCCAGCGATCTCTCCACTGTGCTTTCAGAAGTGC 4200

4201 CCTTCCTGCTGCGAAGTTCTCCCATCCTGGGACGGCGGCAGTATTGAAGCTCGTGACAAG 4260

4261 TGCCTTCACACAGAACCCTCGGAACTGTCCACGCGTTCCGTGGGAACAAGGGGTT 4315
```

Figure 1B
Page 6 of 6

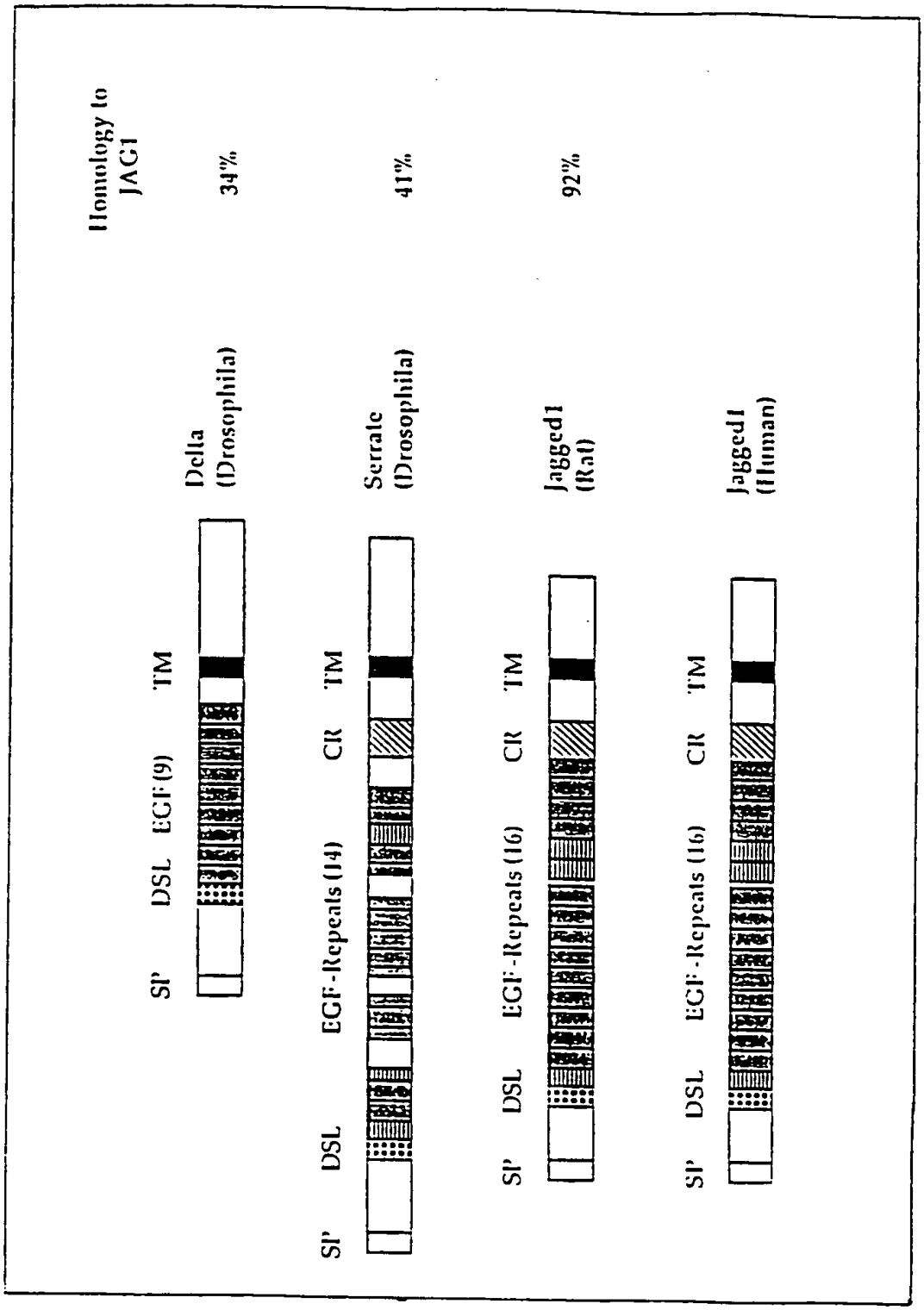


Figure 1C

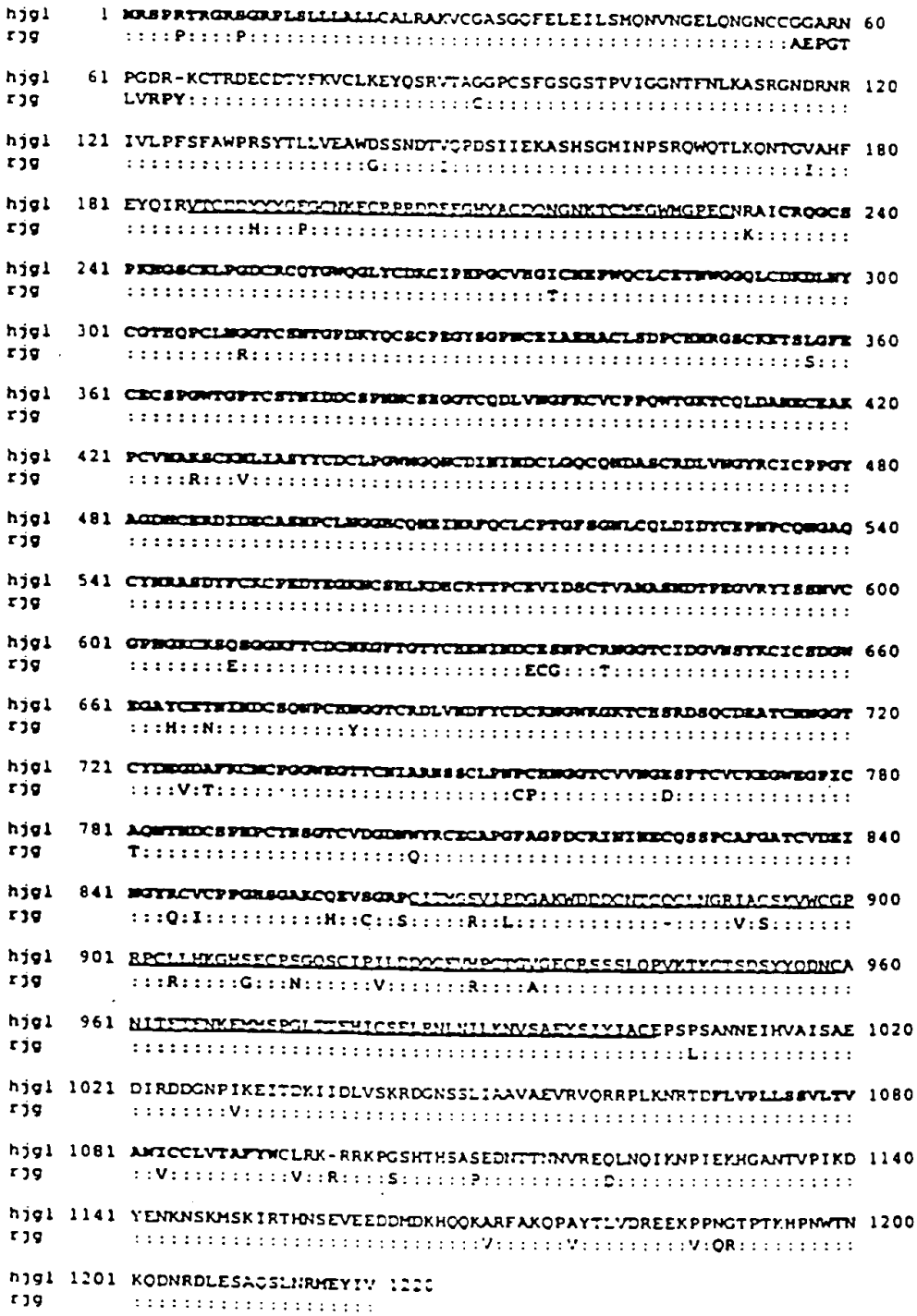


Figure 2A

Figure 2B

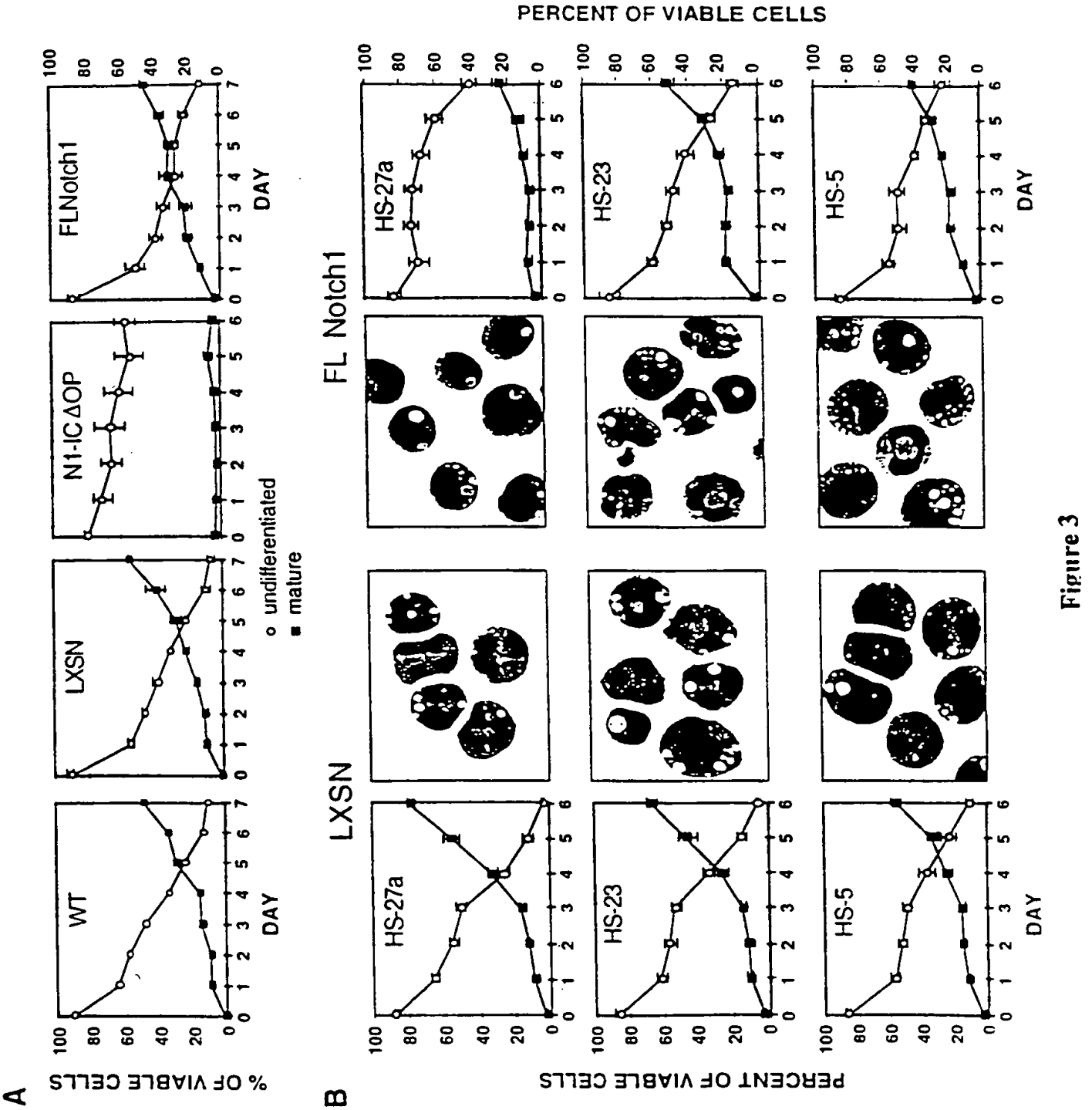

A
% OF VIABLE CELLS
WT
LXSN
N1-ICΔOP
FLNotch1
DAY
o undifferentiated
■ mature
B
PERCENT OF VIABLE CELLS
LXSN
FL Notch1
HS-27a
HS-23
HS-5
DAY

Figure 3

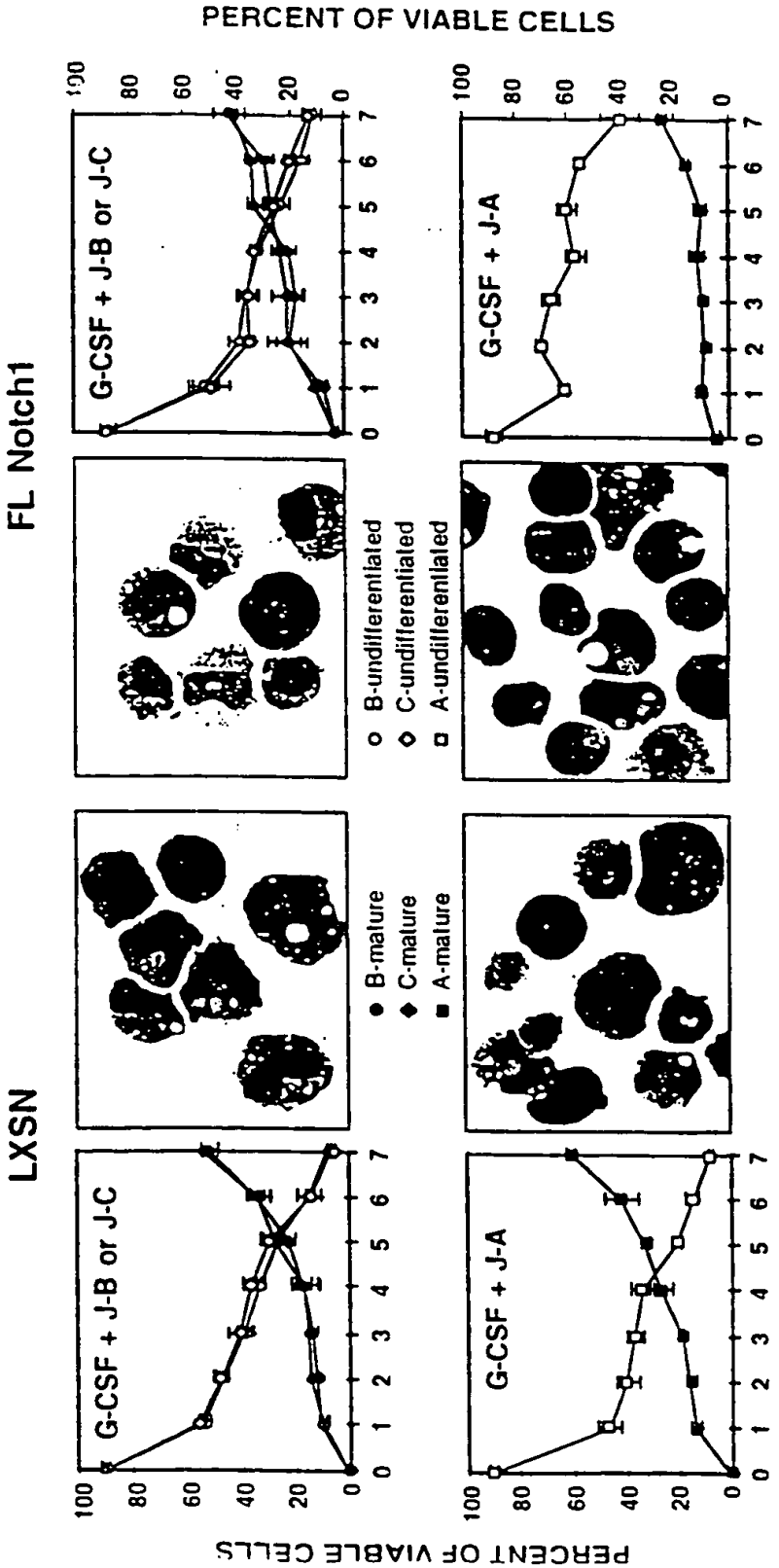

PERCENT OF VIABLE CELLS
FL Notch1
LXSN
G-CSF + J-B or J-C
G-CSF + J-A
B-undifferentiated
C-undifferentiated
A-undifferentiated
B-mature
C-mature
A-mature
0 1 2 3 4 5 6 7
0 20 40 60 80 100

Figure 4

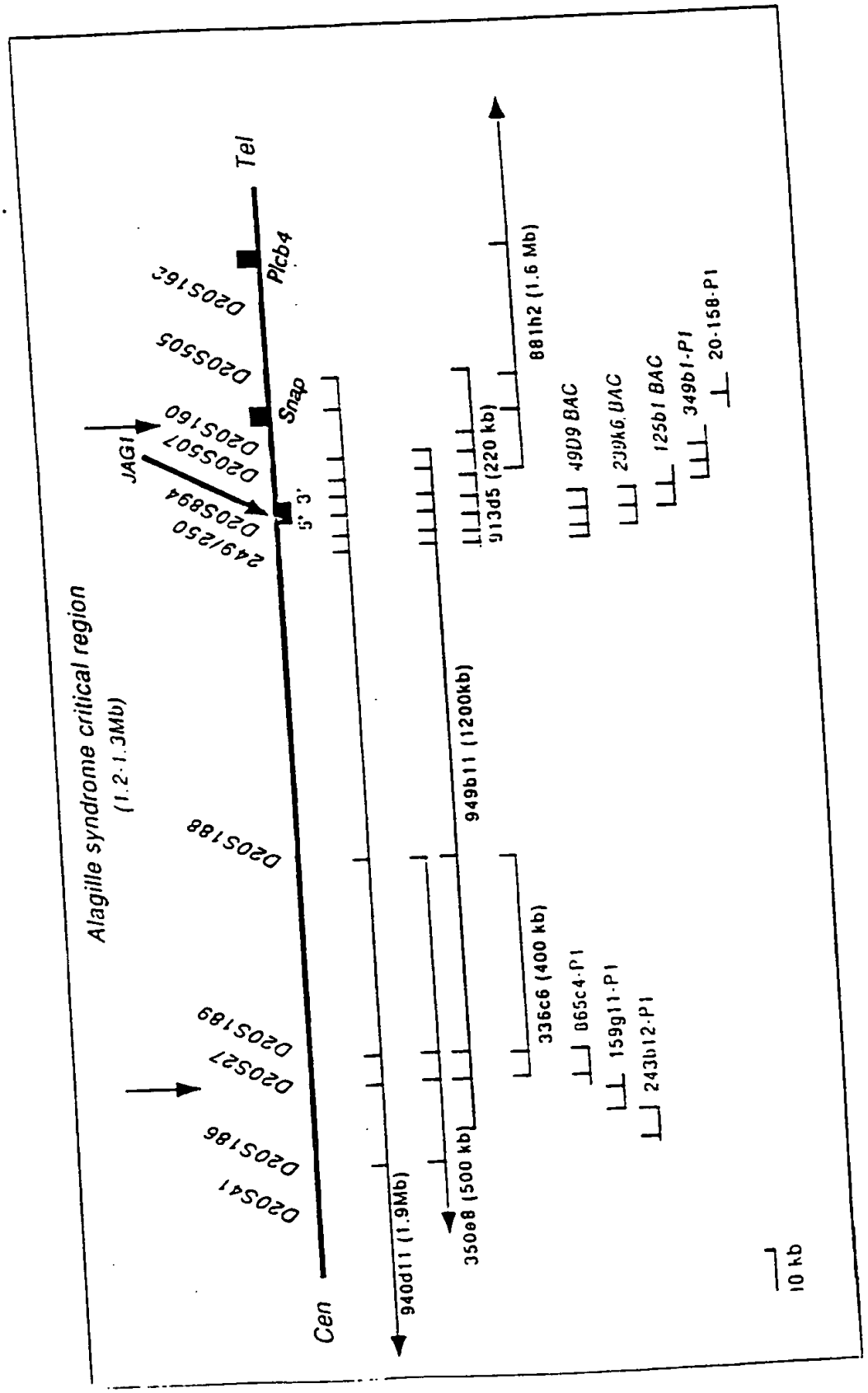
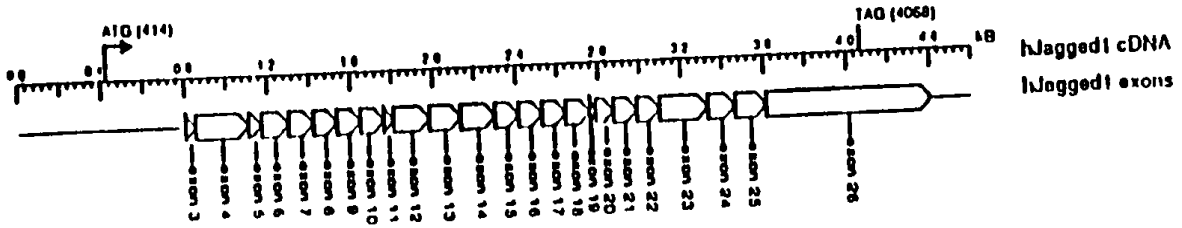

Alagille syndrome critical region
(1.2-1.3Mb)
Tel
Cen
Plcb4
Snap
JAG1
5'
3'
D20S162
D20S505
D20S160
D20S507
D20S894
249/250
D20S188
D20S189
D20S27
D20S186
D20S41
940d11 (1.9Mb)
350e8 (500 kb)
949b11 (1200kb)
913d5 (220 kb)
881h2 (1.6 Mb)
336c6 (400 kb)
865c4-P1
159g11-P1
243b12-P1
490 BAC
239k6 BAC
125b1 BAC
349b1-P1
20-158-P1
10 kb

Figure 5

A



B

Exon Number	Intron/EXON...EXON/Intron	JAG1 cDNA	Exon Length
Exons 1,2	not available	801-852	52
Exon 3	gttcgtgcag/AGGTCCTACA(28).GACACCGTTC/gtcagtatcg(29)	853-1107	255
Exon 4	ttgtctctag/AACCTGACAG(30)TGTAACAGAG/gtatgtgtgt (31)	1108-1168	61
Exon 5	tttttacag/CTATTTGCCG(32).GTGACTGCAG/g (33)	1169-1299	131
Exon 6	gtgtctccag/GTGCCAGTAT(34)TGTGACAAAG/gtatggccct (35)	1300-1419	120
Exon 7	tttttggcag/ATCTCAATTA(36).TGTGAAATTG/gtaagtggtc (37)	1420-1533	114
Exon 8	gttttttgcag/CTGAGCACGC(38).TGCTCTACAA/gtaagtccaa (39)	1534-1647	114
Exon 9	tgttgaccag/ACATTGATGA(40).TGCCAGTTAG/gtaagaacat (41)	1648-1761	114
Exon 10	ctcctttcag/ATGCAAATGA(42).TGTGACATAA/gtgagtgact (43)	1762-1808	47
Exon 11	tatttttttag/ATATTAATGA(44)CTCCTGTCGG/gtatgtaaat (45)	1809-1982	174
Exon 12	cctcttctag/GATTTGGTTA(46)CCTCTGTCAG/gtgagtggtg (47)	1983-2133	151
Exon 13	atatttgcag/CTGGACATCG(48)CCCTGTGAAG/gtacctcccc (49)	2134-2298	165
Exon 14	tatcttctag/TGATTGACAG(50)TGCCATGAAA/gtaagactcc (51)	2299-2412	114
Exon 15	tgtttcatag/ATATTAATGA(52)TGTGAAACCA/aagagtgtgc (53)	2413-2526	114
Exon 16	ttgattctag/ATATTAATGA(54).GGCCACTCAC/gtaagtggta (55)	2527-2640	114
Exon 17	tttcctccag/GTGACAGTCA(56)TGTAACATAG/gtaactttat (57)	2641-2757	117
Exon 18	tcttttatag/CCCGAAACAG(58)TGTGCTCAGA/gtgagtgtcc (59)	2758-2785	28
Exon 19	ttctttgcag/ATACCAATGA(60)CTCATCCCTG/gtaagtgtga (61)	2786-2871	86
Exon 20	gctttcttag/TTACAACAGC(62)TGCAGAATAA/gtaaggactg (63)	2872-2985	114
Exon 21	tttctcctag/ACATCAATGA(64)TGCCAGGAAG/gtatgtgtgc (65)	2986-3095	110
Exon 22	cacctgtcag/TTTCAGGGAG(66)CTGCTCAAAG/gtaggacatg (67)	3096-3329	234
Exon 23	ttttctccag/GTCTGGTGTT(68)GATGTCACCA/gtatgtaaca (69)	3330-3461	132
Exon 24	atcgttttag/GGTCTTACTA(70)TGTGGCCATT/gtaagtataa (71)	3462-3612	151
Exon 25	gtttttccag/TCTGCTGAAG(72)AACAGAACAG/gtaggtgtca (73)	3613-4404+	792+
Exon 26	tgccttacag/ATTTGCTTGT(74)		

Figure 6

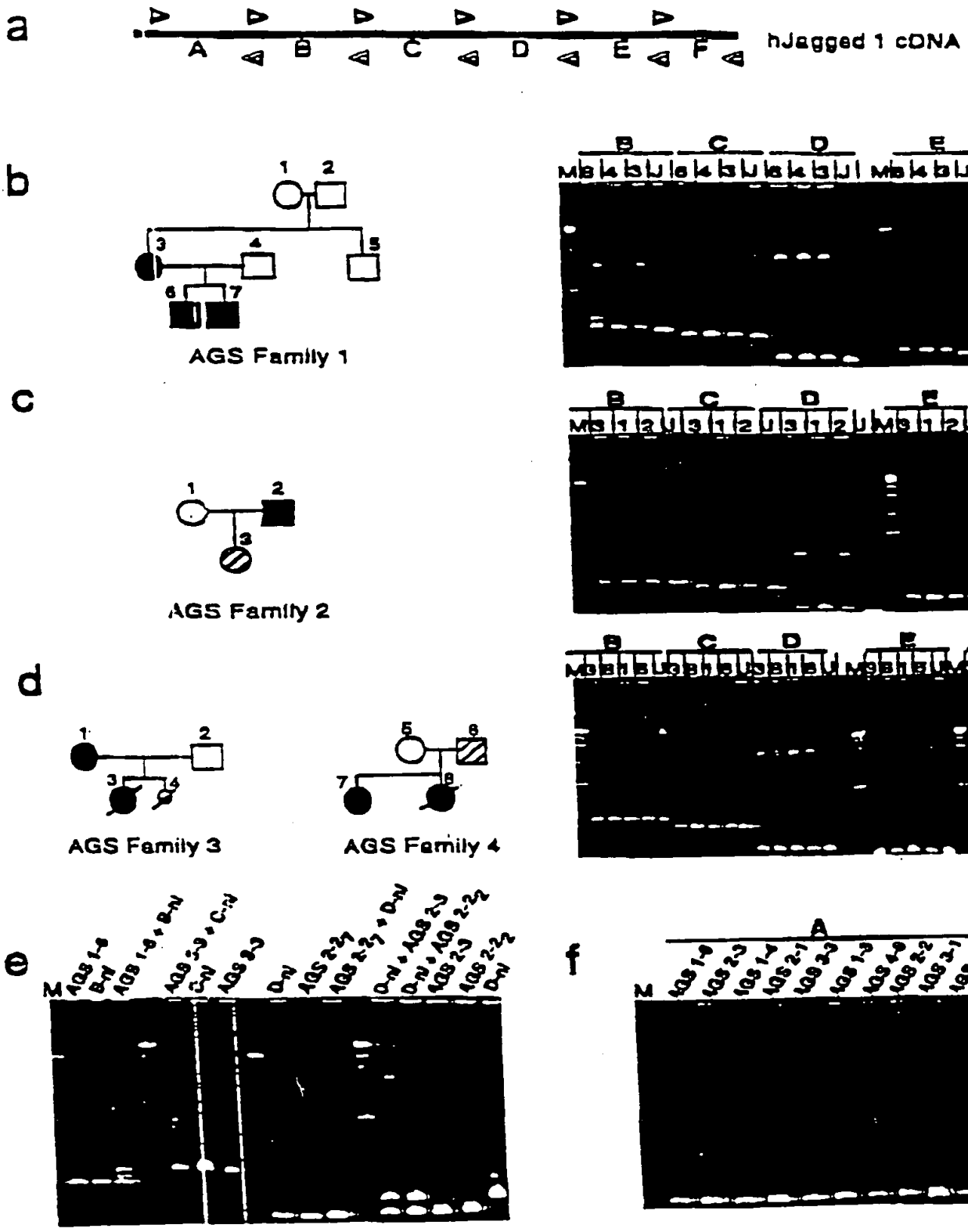

a
A
B
C
D
E
F
hJagged 1 cDNA
b
AGS Family 1
c
AGS Family 2
d
AGS Family 3
AGS Family 4
e
f

Figure 7

CORRECTED VERSION*

PCT

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Filed on 25 June 1997 (25.06.97)

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(74) Agents: GASHLER, Andrea, L. et al.; Campbell & Flores LLP, Suite 700, 4370 La Jolla Village Drive, San Diego, CA 92122 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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(54) Title: HUMAN JAGGED POLYPEPTIDE, ENCODING NUCLEIC ACIDS AND METHODS OF USE

(57) Abstract

The present invention provides a method of inhibiting differentiation of hematopoietic progenitor cells by contacting the progenitor cells with an isolated JAGGED polypeptide, or active fragment thereof. The invention additionally provides a method of diagnosing Alagille Syndrome in an individual. The method consists of detecting an Alagille Syndrome disease–associated mutation linked to a JAGGED locus.

*(Referred to in PCT Gazette No. 18/1999, Section II)

ENSDOCID: <WO 9858958A2_IA>

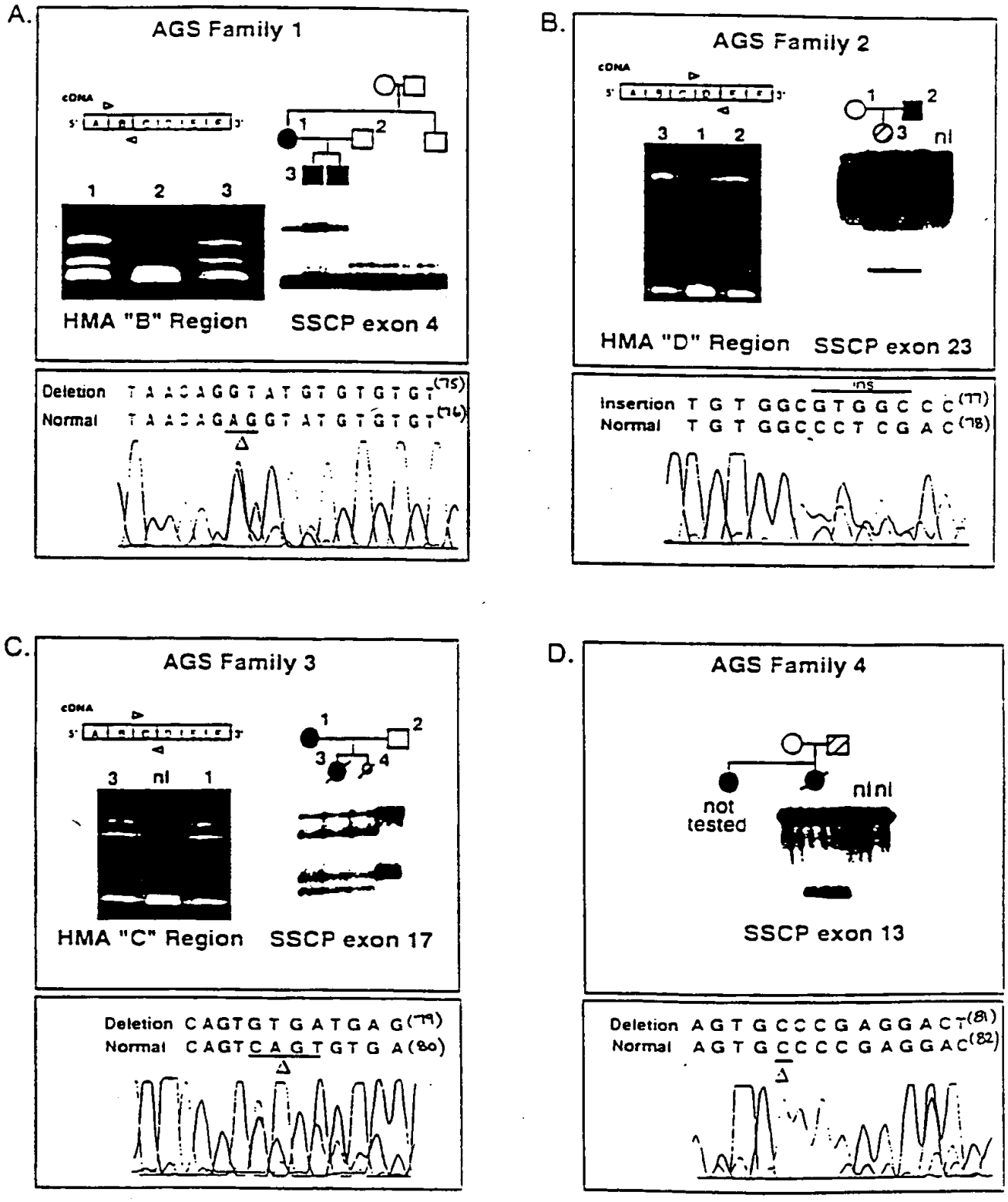

Figure 8

Individuals	cDNA Mutations	EXONS/ Nucleotide Changes	Amino Acid Mutations		Predicted Translation Products
					SP DSL EGF-Repeats CR TM 1 1219
AGS Family 1	1104delAG	Exon 4 del AG	Amino Acid Change After 230	Normal: CN(230)RAICRQGCS Mutant: CN(230)SYLPTRLQS*	1 230 240(stop)
AGS Family 2	3102 ins5	Exon 23 ins GTGGC	Amino Acid Change After 898	Normal: WCG(898)PRPCL... Mutant: WCG(898)VALDL...	1 898 945 (Stop)
AGS Family 3	2531del4	Exon 17 del CAGT	Amino Acid Change After 708	Normal: DS(708)QCD... Mutant: DS(708)VMR...	1 708 741 (Stop)
AGS Family 4	2066delC	Exon 13 del C	Amino Acid Change After 553	Normal: FCKCP(553)ED... Mutant: FCKCP(553)RT...	1 553 563 (Stop)

Figure 9

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INTERNATIONAL SEARCH REPORT

Int ational Application No
PCT/US 98/13207

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 C07K14/47 C12Q1/68 C12N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	EP 0 861 894 A (ASAHI CHEMICAL IND) 2 September 1998 see the whole document	1,2, 4-17, 20-23
X	& WO 97 19172 A (ASAI CHEMICAL IND.) 29 May 1997 see abstract see page 62 - page 76 ---	1,2, 4-17, 20-23
X	WO 96 27610 A (UNIV YALE ;IMP CANCER RES TECH (GB); ISH HOROWICZ DAVID (GB); HENR) 12 September 1996 see abstract see page 79, line 22 - page 86, line 24 --- -/--	1,2

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
26 January 1999	09/02/1999

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LINDSELL ET AL.: "Jagged a mammalian ligand that activates Notch1" CELL, vol. 80, no. 6, 1995, pages 909-917, XP002090589	1,2
A	see the whole document	4-25
X,P	WO 97 45143 A (MONTESANO ROBERTO ;UNIV GENEVE (CH); PEPPER MICHAEL S (CH); MACIAG) 4 December 1997 see abstract see page 6, line 10 - page 9, line 30 see page 54 - page 61 see claims 1,2,48,49	1,2, 4-12, 14-17, 20-23
X,P	ODA T. ET AL.: "Mutations in the human Jagged1 gene are responsible for Alagille syndrome " NATURE GENETICS, vol. 16, no. 3, 1997, pages 235-242, XP002090587 us see the whole document	1,2, 26-30
X,P	ODA T. ET AL.: "Identification and cloning of the human homolog (JAG1) of the rat Jagged1 gene from the Alagille syndrome critical region at 20p12" GENOMICS, vol. 43, no. 3, 1 August 1997, pages 376-379, XP002090588 us see the whole document	1,2, 26-30
X,P	LI L. ET AL.: "Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1" NATURE GENETICS, vol. 16, no. 3, 1997, pages 243-251, XP002090590 us see the whole document	1,26-30

2

INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/US 98/ 13207

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 26-31
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 26-31 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In ational Application No
PCT/US 98/13207

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0861894 A	02-09-1998	AU 7587696 A	11-06-1997
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HUMAN JAGGED POLYPEPTIDE, ENCODING NUCLEIC ACIDS AND METHODS OF USE

This invention was supported by grant numbers P30HD28834, P50HL54881, DK34431, DK51417, CA18221, HL36444, 1R01DK53104-01, DK02338-03 and 5P30HD288215 awarded by the National Institute of Health, USPHS Grant CA58207 and contract DE-AC-03-76SF00098 from the U.S. Department of Energy. The United States Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

This invention relates to polypeptides and peptides for regulating stem cell differentiation and renewal and to the molecular defects involved in Alagille Syndrome.

Hematopoiesis involves a delicate balance between progenitor cell self-renewal and differentiation. Self-renewal generates additional progenitor cells through cell division, and differentiation produces specialized cell types such as red blood cells or lymphocytes. The ability to reliably reproduce hematopoietic differentiation and expansion in vitro would greatly facilitate the development of clinical therapeutic treatments based on blood products and cell transplantation. For example, the ability to modulate hematopoietic differentiation and expansion would promote the production of mature blood cells for transfusion therapies and the production of mature dendritic cells for immunotherapy. In addition, the ability to manipulate a hematopoietic cell population to maintain a large number of progenitor cells would greatly improve *ex vivo* retroviral gene therapy since cell proliferation is required for retroviral gene transduction.

The ability to maintain the survival and proliferation of hematopoietic progenitor cells and to inhibit their differentiation would also improve cell transplantation following tumor purging. In high-dose chemotherapy, doses of toxic drugs are escalated to destroy aggressive malignancies such as hematologic, breast, testicular and ovarian cancers. These high doses also destroy many of the rapidly cycling cells of the hematopoietic system, rendering a patient vulnerable to infection. The ability to promote the survival and expansion of a limited number of remaining hematopoietic progenitor cells would increase neutrophil and platelet recovery times and reduce the danger associated with tumor purging and hematopoietic cell transplantation. However, current technology cannot effectively regulate the balance of hematopoietic progenitor cell survival and differentiation.

During embryogenesis in *Drosophila*, the Notch receptor plays a central role in cell fate specification during development of the central and peripheral nervous systems, eye, mesoderm, wing, bristles and ovaries. The Notch family of cell-cell signaling receptors is highly conserved in fly, worm, frog as well as higher vertebrates, and functions to determine cell fate through the transduction of signals between cells in direct contact with each other.

In higher vertebrates, the process of cell-fate determination is integral to hematopoiesis, where the balance between stem cell or progenitor cell self-renewal and differentiation is carefully regulated. Notch homologues can play a role in determining cell fate in hematopoietic cells, as evidenced by the expression of Notch1 RNA in immature hematopoietic precursor cells from adult human bone marrow. Notch homologues are implicated in T lymphocyte development since the human Notch

homologue, TAN-1 (hNotch1), was isolated from a T-cell leukemia containing a translocation between Notch and the T cell receptor (TCR)-β gene. In addition, Notch1 can influence the CD4/CD8 cell-fate decision. Because an activated form of Notch1 can inhibit G-CSF-induced granulocytic differentiation of 32D myeloid progenitors, Notch also can play a role in mediating cell-fate decisions in the myeloid lineage.

The evolutionary conservation of Notch is reflected in the corresponding conservation of Notch ligands. Several Notch ligands have been identified thus far, including Delta and Serrate in *Drosophila*; LAG-2 and APX-1 in *C. elegans*; X-Delta-1 in *Xenopus*; C-Delta-1 and C-Serrate-1 in the chick; Delta-like-1 (Dll1) in the mouse; and Jagged1 and Jagged2 in the rat. Each of these Notch ligands share two important extracellular features: the DSL domain, defined by a conserved region among Delta Serrate, and LAG-2, and tandem epidermal growth factor (EGF) repeats. Delta and Serrate have been shown to interact with Notch in *Drosophila*, and fibroblasts expressing rat Jagged1 inhibit muscle cell differentiation of Notch1-expressing C2C12 cells. These results indicate that DSL family polypeptides including *Drosophila* Delta and Serrate and rat Jagged can function as Notch ligands.

However, a human Notch ligand, which would be useful in manipulating the balance of hematopoietic progenitor cell renewal and differentiation, has not yet been identified. Thus, there is a need for a human Notch ligand and for methods of using the ligand to maintain and expand hematopoietic progenitor cells to make clinical blood products and progenitor cells for transplantation. The present invention satisfies this

need by providing human JAGGED1 polypeptides and provides related advantages as well.

The invention also relates to Alagille Syndrome, which is an autosomal dominant, developmental disorder affecting the liver, heart, skeleton, eye, face and kidneys. The course and prognosis of Alagille Syndrome, which occurs at a minimum estimated frequency of 1 in 70,000 live births, varies widely. This multi-system disorder traditionally has been defined by a paucity of intrahepatic bile ducts in association with several of the main clinical abnormalities, which are cholestasis, cardiac disease, skeletal abnormalities, ocular abnormalities and a characteristic facial phenotype. Fifteen percent of Alagille Syndrome patients will require liver transplantation, and seven to ten percent of patients will have severe congenital heart disease.

Unfortunately, the available therapies for Alagille Syndrome are few, and both diagnosis and treatment have been hampered by a lack of knowledge regarding the molecular defect underlying the disease. In a relatively small number of patients, gross chromosomal deletions of chromosome 20 appear to be inherited with the disorder. However, for the large majority of patients lacking such gross chromosomal abnormalities, the genetic defect responsible for Alagille Syndrome has eluded discovery. Identification of the molecular defect responsible for Alagille Syndrome would be useful in the early diagnosis and prenatal testing of individuals at risk for the disorder. In addition, knowledge of mutations resulting in Alagille Syndrome would facilitate the development of new therapies for treating the disorder. Thus, there is a need for identifying the mutations responsible for Alagille Syndrome and for methods of diagnosing the

disorder by analyzing the genetic defect responsible for the disorder. The present invention satisfies this need and also provides related advantages.

SUMMARY OF THE INVENTION

The present invention provides an isolated polypeptide exhibiting substantially the same amino acid sequence as JAGGED, or an active fragment thereof, provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. The invention further provides an isolated nucleic acid molecule containing a nucleotide sequence encoding substantially the same amino acid sequence as JAGGED, or an active fragment thereof, provided that the nucleotide sequence does not encode the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. Also provided herein is a method of inhibiting differentiation of hematopoietic progenitor cells by contacting the progenitor cells with an isolated JAGGED polypeptide, or active fragment thereof. The invention additionally provides a method of diagnosing Alagille Syndrome in an individual. The method consists of detecting an Alagille Syndrome disease-associated mutation linked to a JAGGED locus.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. (A) Nucleotide sequence SEQ ID NO:1 and amino acid sequence SEQ ID NO:2 of the human JAGGED1 (hJAGGED1) cDNA. (B) Partial nucleotide sequence SEQ ID NO:3 and amino acid sequence SEQ ID NO:4 of the human Jagged 2 (hJAGGED2) cDNA. (C) Diagram showing the protein structure of hJAGGED1 in alignment with the *Drosophila* Delta, *Drosophila* Serrate and rat Jagged1 proteins. The signal peptide region is indicated SP. DSL is a domain unique to Notch ligands, shared by

Drosophila Delta and Serrate and the *C. elegans* protein LAG-2. Also indicated are the epidermal growth factor-like repeats (EGF-like repeats); cysteine-rich region (CR) and transmembrane domain (TM). The percent amino acid identity to hJAGGED1 is shown at the right.

Figure 2. (A) Alignment of hJAGGED1 (hJg1) and rJagged1 (rJg) amino acid sequences. The peptide signal sequence (residues 1 to 21), EGF-like repeats (residues 234 to 862), and transmembrane domain (residues 1077 to 1091) are shown in bold type. The DSL domain (residues 185 to 239) and the cysteine-rich region (residues 863 to 1012) are underlined. (B) Alignment of rat Jagged1 amino acid sequence SEQ ID NO:5 and rat Jagged2 amino acid sequence SEQ ID NO:6.

Figure 3. Inhibition of granulocytic differentiation by the hJAGGED1-expressing stromal cell line, HS-27a. (A) Granulocytic differentiation of 32D myeloid progenitor cells in response to granulocyte colony stimulating factor (G-CSF). The parental 32D cell line (WT) and 32D cells transduced with control LXSN retrovirus or retrovirus containing full-length murine Notch1 cDNA (FL Notch1) were evaluated for granulocytic differentiation in response to G-CSF. The relative percentages of cells remaining undifferentiated (o) or showing morphologic characteristics of mature granulocytes (■) are shown; cells showing some characteristics of differentiation, but which were less mature than band cells were excluded from this analysis. This figure shows results obtained concurrently with those depicted in Figure 4 and represents one of three experiments with comparable results. Plots for the LXSN control clones and the FL Notch1 clones each represent the average obtained for three clones with error bars indicating the SEM. The data for 32D cells expressing the activated Notch1 construct, N1-ICΔOP were obtained on

a separate occasion and represent the averages and SEM of six independent clones. (B) Granulocytic differentiation of 32D cells in the presence of G-CSF when cultured on the human stromal cells line HS-27a, HS-23 or HS-5. The results depicted represent data from three separate experiments, each including three LXSN and three FL Notch1 clones as well as the parental 32D line (not shown). Each plot therefore represents the average and SEM of nine values. The center panels show representative Wright stained cells after four days in culture; the same two clones, LXSN-10 and FLN2.4, are depicted in each set of panels.

Figure 4. Inhibition of granulocytic differentiation by a soluble peptide corresponding to part of the hJAGGED1 DSL domain. 32D clones carrying the control LXSN retroviral vector alone or the vector containing FL Notch1 were evaluated for differentiation in the presence of G-CSF and different peptides corresponding to distinct portions of hJAGGED1. Peptide SEQ ID NO:9 ("J-A") corresponds to a portion of the extracellular DSL domain. Peptide SEQ ID NO:10 ("J-B") corresponds to EGF-repeat 1, and peptide SEQ ID NO:11 ("J-C") corresponds to the intracellular domain. Shown is an experiment using 10 μM peptide. Each plot represents the average and SEM of three independent clones. The center panels show representative Wright stained cells (clones LXSN-10 and FL N 2.4) after 6 days in culture with G-CSF and peptide SEQ ID NO:10 (J-B; top panel) or peptide SEQ ID NO:9 (J-A; lower panel).

Figure 5. Mapping hJAGGED1 in the Alagille Syndrome critical region. The critical region has been defined by the shortest region of overlap of patients with deletions of 20p12 by molecular and FISH mapping and extends between P-1 243b12, proximal to D20S27, and clone 20p1-158, proximal to WI-6063. YAC clones are indicated

in standard print, P1 clones are indicated as such, and BAC clones are in italic print.

Figure 6. (A) Schematic diagram illustrating the alignment of the exon boundaries with the hJAGGED1 cDNA sequence. (B) The exon/intron boundary nucleotide sequences are shown for twenty-four hJAGGED1 exons; sequence identification numbers are indicated in parenthesis. One or more 5' exons have not been identified; the 5' most exon identified to date is indicated exon (n+1). The hJAGGED1 cDNA nucleotide positions corresponding to each exon and the exon length are also indicated.

Figure 7. Heteroduplex Mobility Analysis (HMA) of hJAGGED1 cDNAs in four Alagille Syndrome (AGS) families. (A) A schematic diagram showing the positions of the primers used in RT-PCR, and the amplified cDNA regions A through F. (B) HMA of three members of AGS family 1. PCR product amplified from the hJAGGED1 cDNA clone is shown as a reference (lane J). (C) Analysis of three members of AGS family 2. (D) Analysis of two affected members of AGS family 3 and 4. (E) Analysis of cloned cDNA fragments, each containing one variant. Normal clones from region B, C and D are indicated as B-nl, C-nl and D-nl, respectively. (F) HMA of the hJAGGED1 cDNA region A of 10 individuals from AGS families 1-4, showing no heteroduplex formation.

Figure 8. Segregation of SSCP variants in four Alagille Syndrome families. Individuals with filled circles meet full criteria for diagnosis with Alagille syndrome. Individuals with hatched circles have some of the characteristics of the syndrome. (A) Segregation of an exon (n+2) variant in two children with liver, heart, eye and facial features of Alagille Syndrome and their mildly affected mother. Sequence analysis demonstrates a

2 bp "AG" deletion. (B) Segregation of an exon n+21 variant in a child with Alagille facies and pulmonic stenosis and her more severely affected father. Sequence analysis demonstrates a 5 bp insertion (GTGGC) in father and daughter. (C) Family 3 demonstrates an exon (n+15) variant in an affected mother, her affected daughter and DNA from a terminated pregnancy. Sequence analysis demonstrates a 4 bp deletion in affected individuals. (D) Family 4 has an exon 15 variation in a child with severe cardiac and liver disease who died at 5 years of age and her less severely affected father. Sequence analysis in father and daughter demonstrated a single nucleotide "C" deletion. Sequence identification numbers are indicated in parenthesis.

Figure 9. Summary of the mutations identified in Alagille Syndrome individuals and the corresponding predicted translation products. For each of four Alagille Syndrome mutations, the position of the mutation within the hJAGGED1 cDNA and gene are provided, as well as the predicted amino acid mutations and size of the truncated hJAGGED1 polypeptide.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to the discovery of human Notch ligands, designated JAGGED. The polypeptides of the invention are transmembrane proteins that share several structural features with other Notch ligands, including a DSL (Delta/Serrate/Lag-2) domain characteristic of these ligands and tandem epidermal growth factor (EGF)-like repeats. Provided herein are exemplary JAGGED polypeptides, human JAGGED1 (hJAGGED1) and human JAGGED2 (hJAGGED2). hJAGGED1 is expressed in bone marrow stromal cells, and a stromal cell line expressing hJAGGED1 permits survival and proliferation of hematopoietic progenitor cells expressing Notch but

inhibits granulocytic differentiation. As disclosed herein, a JAGGED-derived peptide can mimic the function of an intact JAGGED molecule by inhibiting the differentiation of Notch-expressing progenitor cells (Example II). Thus, the JAGGED polypeptides and peptides of the invention can be used, for example, in *ex vivo* therapy for inhibiting differentiation and maintaining the proliferative potential of progenitor cells such as hematopoietic stem cells.

Thus, the present invention provides an isolated JAGGED polypeptide. An isolated JAGGED polypeptide of the invention can have substantially the same amino acid sequence as the hJAGGED1 sequence SEQ ID NO:2 shown in Figure 1A or substantially the same amino acid sequence as the hJAGGED2 sequence SEQ ID NO:4 shown in Figure 1B, provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6.

As used herein, the term "JAGGED" means a JAGGED polypeptide and includes polypeptides having substantially the same amino acid sequence as the hJAGGED1 polypeptide (SEQ ID NO:2) shown in Figure 1A or the hJAGGED2 polypeptide (SEQ ID NO:4) shown in Figure 1B, provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. hJAGGED1 exhibits an apparent molecular weight of about 150 kDa on SDS-PAGE and is a 1219 amino acid polypeptide having the sequence shown in Figure 1A. As illustrated in Figure 1C, hJAGGED1 is a membrane-bound ligand with a large extracellular domain and a very short intracellular domain. The hJAGGED1 polypeptide shares structural features with the *Drosophila* polypeptides Delta and Serrate and with the rat Jagged1 polypeptide (see Figure 1C). In particular, hJAGGED1 has a DSL domain, which is a region conserved among the Notch ligands Delta, Serrate and LAG-2. In addition, the extracellular domain of

hJAGGED1 contains EGF repeats. A cysteine-rich domain is also present in hJAGGED1, as in Serrate and rat Jagged1. The DSL and EGF-repeat domains can be involved in interaction with the Notch receptor (Henderson et al., Devel. 120:2913-2924 (1994); Lieber et al., Neuron 9:847-859 (1992); and Rebay et al., Cell 67:687-699 (1991), each of which are incorporated herein by reference).

hJAGGED2 is a polypeptide of more than 1150 amino acids and includes the amino acid sequence shown in Figure 1B. Like hJAGGED1, hJAGGED2 is a membrane-bound ligand with a large extracellular domain and a relatively short intracellular domain. The hJAGGED2 polypeptide also has a DSL domain, 15 EGF-like repeats and a transmembrane domain characteristic of membrane-bound Notch ligands.

As disclosed in Example I, hJAGGED1 is widely expressed in a variety of human tissues. However, in bone marrow, hJAGGED1 expression is restricted to a subpopulation of stromal cells. hJAGGED1 is also expressed in the HS-27a cell line, which is a line of spindle-shaped human stromal cells that do not support differentiation of hematopoietic progenitor cells but support the maintenance of immature progenitors for five to eight weeks. The expression of hJAGGED1 in these cells is consistent with a role for JAGGED polypeptides in regulating hematopoietic progenitor cell survival and differentiation.

Co-culture of myeloid progenitor 32D cells expressing full-length Notch with HS-27a cells, which express hJAGGED1, inhibits G-CSF induced granulocytic differentiation of the 32D cells (see Example II). As disclosed herein, a peptide corresponding to part of the hJAGGED1 DSL domain (residues 188 to 204; SEQ ID NO:9)

also inhibits differentiation of Notch-expressing 32D cells in the presence of G-CSF. Thus, the present invention provides JAGGED polypeptides and peptides useful for maintaining the proliferative potential and inhibiting differentiation of progenitor cells such as hematopoietic progenitor cells.

The term JAGGED encompasses a polypeptide having the sequence of the naturally occurring hJAGGED1 polypeptide (SEQ ID NO:2) or the sequence of the naturally occurring hJAGGED2 polypeptide (SEQ ID NO:4) and is intended to include related polypeptides having substantial amino acid sequence similarity to hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4), provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. Such related polypeptides exhibit greater sequence similarity to hJAGGED1 or hJAGGED2 than to other DSL-containing polypeptides or EGF-repeat containing polypeptides and include alternatively spliced forms of hJAGGED1 or hJAGGED2 and isotype variants of the amino acid sequences shown in Figure 1A and 1B, provided that the polypeptides do not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. The hJAGGED1 and hJAGGED2 polypeptides disclosed herein have about 54% identity to each other at the amino acid level. As used herein, the term JAGGED describes polypeptides generally having an amino acid sequence with greater than about 50% identity, preferably greater than about 60% identity, more preferably greater than about 70% identity, and can be a polypeptide having greater than about 80%, 90%, 95%, 97%, or 99% amino acid sequence identity with hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4), provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6.

A JAGGED polypeptide can be more closely related to hJAGGED1, for example, than to hJAGGED2. Thus, a JAGGED polypeptide can be a member of the JAGGED1 subfamily or a member of the JAGGED2 subfamily. A member of the JAGGED1 subfamily is a polypeptide having substantially the same amino acid sequence as hJAGGED1 (SEQ ID NO:2), or an active fragment thereof, provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5. A member of the JAGGED1 subfamily generally has an amino acid sequence with greater than about 50% identity, preferably greater than about 60% identity, more preferably greater than about 70% identity, and can be a polypeptide having greater than about 80%, 90%, 95%, 97%, or 99% amino acid identity with hJAGGED1 (SEQ ID NO:2), provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5.

Similarly, a member of the JAGGED2 subfamily is a polypeptide having substantially the same amino acid sequence as hJAGGED2 (SEQ ID NO:4), or an active fragment thereof, provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:6. A member of the JAGGED2 subfamily generally has an amino acid sequence with greater than about 50% identity, preferably greater than about 60% identity, more preferably greater than about 70% identity, and can be a polypeptide having greater than about 80%, 90%, 95%, 97%, or 99% amino acid identity with hJAGGED2 (SEQ ID NO:4), provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:6.

As used herein, the term "substantially the same amino acid sequence," when used in reference to a JAGGED amino acid sequence, is intended to mean the sequence shown in Figure 1A or Figure 1B, or a similar, non-identical sequence that is considered by those

skilled in the art to be a functionally equivalent amino acid sequence, provided that the amino acid sequence is not the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. For example, an amino acid sequence that has substantially the same amino acid sequence as JAGGED can have one or more modifications such as amino acid additions, deletions or substitutions relative to the amino acid sequence of hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4), provided that the modified polypeptide retains substantially at least one biological activity of hJAGGED1 or hJAGGED2, such as substantially the ability to bind and activate a Notch receptor or substantially the ability to inhibit progenitor cell differentiation, provided that the modified polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. Comparison of sequences for substantial similarity can be performed between two sequences of any length and usually is performed with nucleotide sequences of between 5 and 3500 nucleotides, preferably between about 10 and 300 nucleotides and more preferably between about 15 and 50 nucleotides. Comparison for substantial similarity between amino acid sequences is usually performed with sequences between about 6 and 1200 residues, preferably between about 10 and 100 residues and more preferably between about 25 and 35 residues. Such comparisons for substantial similarity are performed using methodology routine in the art.

Therefore, it is understood that limited modifications can be made without destroying the biological function of a JAGGED polypeptide and that only a portion of the entire primary sequence can be required in order to effect activity. For example, minor modifications of hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4) that do not destroy polypeptide activity also fall within the definition of JAGGED and within the definition of the polypeptide claimed as such, provided

that such modifications do not produce a polypeptide having the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. Also, for example, genetically engineered fragments of JAGGED either alone or fused to heterologous proteins such as fragments or fusion proteins that retain measurable activity in binding and activating Notch or a Notch homologue, in inhibiting progenitor cell differentiation, or other inherent biological activity of JAGGED fall within the definition of the polypeptide claimed as such.

It is understood that minor modifications of primary amino acid sequence can result in polypeptides which have substantially equivalent or enhanced function as compared to the hJAGGED1 sequence set forth in Figure 1A or the hJAGGED2 sequence set forth in Figure 1B. These modifications can be deliberate, as through site-directed mutagenesis, or can be accidental such as through mutation in hosts harboring a JAGGED encoding nucleic acid. All such modified polypeptides are included in the definition of a JAGGED polypeptide as long as at least one biological function of JAGGED is retained, provided that the polypeptide does not have the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. Further, various molecules can be attached to a JAGGED polypeptide including, for example, other polypeptides, carbohydrates, lipids, or chemical moieties. Such modifications are included within the definition of a JAGGED polypeptide.

Several Notch ligands have been identified including ligands from *Drosophila*, *C. elegans*, *Xenopus*, mouse and rat. Known Notch ligands include Delta and Serrate in *Drosophila* (Baker et al., Science 250:1370-1377 (1990); Cuoso et al., Cell 67:311-323 (1994)); LAG-2 and APX-1 in *C. elegans* (Mello et al.,

Cell 77:95-106 (1994); Tax et al., Nature 368:150-154 (1994); Henderson et al., Develop. 120:2913-2924 (1994)); X-Delta-1 in *Xenopus* (Chitnis et al., Nature 375:761-766 (1995)); C-Delta-1 (Henrique et al., 1995) and C-Serrate-1 in the chick (Myat et al., Dev. Biol. 174:233-247 (1996); Delta-like-1 (Dll1) in the mouse (Bettenhausen et al., Devel. 121:2407-2418 (1995)); and Jagged1 and Jagged2 in the rat (Lindsell et al., Cell 80:909-917 (1995); Shawber et al., Dev. Biol. 370-376 (1996)). However, these Notch ligands are not JAGGED polypeptides as defined herein. The rat Jagged1 polypeptide (SEQ ID NO:5) and rat Jagged2 polypeptide (SEQ ID NO:6) are explicitly excluded from the definition of a JAGGED polypeptide as defined herein. Other Notch ligands described above, which may share the ability to activate Notch or a Notch homologue, lack substantial amino acid sequence similarity with hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4) and, thus, are not JAGGED polypeptides as defined herein.

In one embodiment, the invention provides an isolated JAGGED polypeptide including substantially the same amino acid sequence as JAGGED, or an active fragment thereof, provided that said polypeptide does not have the amino acid sequence of SEQ ID NO:5, the amino acid sequence of SEQ ID NO:6, the amino acid sequence designated by GenBank accession number U61276, the amino acid sequence designated by GenBank accession number U77720, or the amino acid sequence designated by GenBank accession number U77914.

The present invention also provides active fragments of a JAGGED polypeptide. As used herein, the term "active fragment" means a polypeptide fragment having substantially the same amino acid sequence as a portion of a JAGGED polypeptide, provided that the JAGGED

fragment retains at least one biological activity of JAGGED. An active fragment can have, for example, substantially the same amino acid sequence as a portion of hJAGGED1 (SEQ ID NO:2) or substantially the same amino acid sequence as a portion of hJAGGED2 (SEQ ID NO:4). A biological activity of JAGGED can be, for example, the ability to bind and activate Notch or a Notch homologue, the ability to inhibit differentiation of a hematopoietic progenitor cell or the ability to maintain or increase the proliferative potential of a hematopoietic progenitor cell. Examples of active fragments are provided herein as SEQ ID NO:7, which is a soluble active fragment of hJAGGED1 containing residues 1 to 1010, and SEQ ID NO:8, which is a soluble active fragment of hJAGGED1 containing residues 178 to 240. As disclosed in Example II, these soluble JAGGED fragments have activity in inhibiting granulocytic differentiation of primary mouse hematopoietic cells or in increasing their proliferative potential. Explicitly excluded from the definition of an active fragment are polypeptide portions of SEQ ID NO:5 and SEQ ID NO:6.

The term "isolated," as used herein in reference to a polypeptide, peptide or nucleic acid molecule of the invention, means a polypeptide, peptide or nucleic acid molecule that is in a form that is relatively free from contaminating lipids, polypeptides, nucleic acids or other cellular material normally associated with the polypeptide, peptide or nucleic acid molecule in a cell.

A modified JAGGED polypeptide, or fragment thereof, can be assayed for activity using one of the assays described in Example II or using another assay for measuring progenitor cell differentiation or the maintenance of proliferative potential known in the art. For example, a retroviral expression vector containing a

nucleic acid molecule encoding a modified hJAGGED1 or hJAGGED2 polypeptide, or fragment thereof, can be introduced into HS-23 cells, and the transduced cells assayed for the ability to inhibit differentiation of progenitor cells, such as 32D myeloid progenitor cells expressing full-length Notch, in the presence of a differentiating agent such as G-CSF. A soluble JAGGED polypeptide or fragment thereof can be assayed, for example, by introducing an expression vector containing a nucleic acid molecule encoding the soluble JAGGED polypeptide or fragment into a cell and subsequently assaying the culture supernatant for the ability to inhibit hematopoietic progenitor cell differentiation as described in Example II.

The nucleic acid to be assayed can encode an amino acid sequence corresponding to a portion of native hJAGGED1 (SEQ ID NO:2) or native hJAGGED2 (SEQ ID NO:4) or can be modified to encode one or more amino acid substitutions, deletions or insertions, provided that the nucleic acid molecule does not encode the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. One or more point mutations can be introduced into the nucleic acid encoding the modified JAGGED polypeptide or fragment to be assayed using, for example, site-directed mutagenesis (see Wu (Ed.), Meth. In Enzymol. Vol. 217, San Diego: Academic Press (1993); Chapter 22 of Innis et al. (Ed.), PCR Protocols, San Diego: Academic Press, Inc. (1990), each of which is incorporated herein by reference). Such mutagenesis can be used to introduce a specific, desired amino acid substitution, deletion or insertion; alternatively, a nucleic acid sequence can be synthesized having random nucleotides at one or more predetermined positions to generate random amino acid substitutions. Scanning mutagenesis also can be useful in generating nucleic acid molecules encoding JAGGED polypeptides or fragments that are modified throughout the entire

polypeptide or fragment sequence. Such modified fragments can be screened for the ability to inhibit Notch-expressing 32D cell differentiation as described in Example II; for the ability to increase the self-renewal capacity of hematopoietic progenitor cells (Example II); or using another assay for measuring progenitor cell differentiation or the maintenance of progenitor cell proliferative potential that is known in the art.

If desired, a pool of modified JAGGED polypeptides or JAGGED fragments can be assayed for activity *en masse*. For example, to identify an active fragment of hJAGGED1 or hJAGGED2, pools of synthetic JAGGED fragments or pools of cell supernatants can be assayed for the ability to inhibit the differentiation of 32D cells expressing Notch; subsequently, pools of fragments or supernatants with activity can be subdivided, and the assay repeated in order to isolate the active modified hJAGGED1 or hJAGGED2 polypeptide or fragment from the active pool.

An isolated JAGGED polypeptide, or active fragment thereof, can be obtained by a variety of methods known within the art, including biochemical, recombinant and chemical synthesis methods. Biochemical methods for isolating a JAGGED polypeptide, or active fragment thereof, include preparative gel electrophoresis, gel filtration, affinity chromatography, ion exchange and reversed phase chromatography, chromatofocusing, isoelectric focusing and sucrose or glycerol density gradients (see, for example, Chapter 38 of Deutscher, Methods in Enzymology: Guide to Protein Purification, Vol. 182, Academic Press, Inc., San Diego (1990) and Chapter 8 of Balch et al., Methods in Enzymology, Vol. 257, Academic Press, Inc., San Diego (1995), each of which is incorporated herein by reference in its entirety). For example, as disclosed herein in

Example I, hJAGGED1 RNA is expressed in a variety of human tissues, including stomach, thyroid, spinal cord, lymph node, trachea, adrenal gland, and bone marrow, and in the human bone marrow stromal cell line HS-27a (Roecklein and Torok-Storb, Blood 85:997-1005 (1995), which is incorporated herein by reference). From these results, one skilled in the art knows that one of these tissues or the HS-27a cell line can be used as a source of material for isolating a hJAGGED1 polypeptide.

Preparative gel electrophoresis can be useful in preparing an isolated JAGGED polypeptide or active fragment of the invention. For example, a JAGGED polypeptide, or active fragment thereof, can be isolated by preparative polyacrylamide gel electrophoresis and elution of the polypeptide or fragment by diffusion or electroelution (see, for example, Chapter 33 of Deutscher, *supra*, 1990). Continuous elution gel electrophoresis using a system such as the Model 491 Prep Cell (BioRad, Hercules, CA) can be used to purify a JAGGED polypeptide, or active fragment thereof. If desired, continuous elution gel electrophoresis can be combined with further purification steps such as liquid phase preparative isoelectric focusing using, for example, the Rotofor system (BioRad).

Affinity chromatography is particularly useful in preparing an isolated JAGGED polypeptide or active fragment of the invention. A polypeptide that interacts with a JAGGED polypeptide, for example, a Notch polypeptide, can be useful as an affinity matrix for isolating a JAGGED polypeptide or active fragment of the invention. One skilled in the art understands that polypeptide fragments such as fragments of Notch also can be useful affinity matrices for isolating a JAGGED polypeptide or active fragment of the invention.

Immunoaffinity chromatography can be particularly useful in isolating a JAGGED polypeptide or active fragment thereof. For example, immunoprecipitation or column chromatography with an antibody that selectively binds JAGGED can be used to isolate a JAGGED polypeptide or active fragment thereof. An anti-JAGGED monoclonal or polyclonal antibody that selectively binds JAGGED can be prepared using an immunogen such as the sequence shown as SEQ ID NO:2, or a synthetic peptide fragment thereof, as described further below. One skilled in the art understands that a particularly useful immunogen can be a synthetic peptide fragment of SEQ ID NO:2 or SEQ ID NO:4 having a sequence that is relatively unique to JAGGED. Thus, in selecting an immunogen, one can exclude, if desired, regions of SEQ ID NO:2 or SEQ ID NO:4 which are conserved among other proteins. Methods of affinity chromatography are well known in the art and are described, for example, in Chapters 29, 30 and 38 of Deutscher, *supra*, 1990, which has been incorporated herein by reference.

Recombinant methods for producing a polypeptide through expression of a nucleic acid sequence in a suitable host cell also are well known in the art and are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed, Vols 1 to 3, Cold Spring Harbor Laboratory Press, New York (1989), which is incorporated herein by reference. Nucleic acids for expression of a JAGGED polypeptide are provided herein as SEQ ID NO:1 and SEQ ID NO:3. The production of recombinant hJAGGED1 polypeptide is illustrated in Example II.

A recombinant JAGGED polypeptide or active fragment of the invention can be expressed as a fusion protein with a heterologous "tag" for convenient

isolation from bacterial or mammalian host proteins. For example, histidine-tagged recombinant JAGGED can be isolated by nickel-chelate chromatography. Similarly, a glutathione-S-transferase tag or an antigenic tag such as "FLAG," "AU" or a myc epitope tag also can be included in a recombinant JAGGED polypeptide or active fragment of the invention (Sambrook et al., *supra*, 1989). The use of the PinPoint™ expression system for expression of the hJAGGED1 active fragment SEQ ID NO:8 as a fusion protein with a heterologous biotinylated peptide is illustrated in Example II.

A JAGGED polypeptide fragment or a JAGGED peptide of the invention can be produced by chemical synthesis, for example, by the solid phase peptide synthesis method of Merrifield et al., J. Am. Chem. Soc. 85:2149 (1964), which is incorporated herein by reference. Standard solution methods well known in the art also can be used to synthesize a peptide or polypeptide fragment useful in the invention (see, for example, Bodanszky, Principles of Peptide Synthesis, Springer-Verlag, Berlin (1984) and Bodanszky, Peptide Chemistry, Springer-Verlag, Berlin (1993), each of which is incorporated herein by reference). A newly synthesized peptide or polypeptide fragment can be purified, for example, by high performance liquid chromatography (HPLC) and can be characterized using mass spectrometry or amino acid sequence analysis.

A JAGGED polypeptide of the invention is useful for preparing an antibody that selectively binds a JAGGED polypeptide such as hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4). An antibody that selectively binds a JAGGED polypeptide can be useful, for example, in purifying a JAGGED polypeptide by immunoaffinity chromatography. Such an antibody also can be useful in diagnosing Alagille Syndrome in an individual by

detecting reduced expression of a JAGGED polypeptide or by detecting an abnormal JAGGED gene product such as a truncated hJAGGED1 gene product. A particularly useful diagnostic antibody can be, for example, an antibody that selectively binds a C-terminal epitope of hJAGGED1, such that the amount of full-length hJAGGED1 polypeptide in a sample can be analyzed.

As used herein, the term antibody is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as polypeptide fragments of antibodies that retain selective binding activity for a JAGGED polypeptide of at least about 1×10^5 M^{-1}. One skilled in the art would know that anti-JAGGED antibody fragments such as Fab, $F(ab')_2$ and Fv fragments can retain selective binding activity for a JAGGED polypeptide and, thus, are included within the definition of an antibody. In addition, the term antibody as used herein includes naturally occurring antibodies as well as non-naturally occurring antibodies and fragments that have binding activity such as chimeric antibodies or humanized antibodies. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis or produced recombinantly. Such non-naturally occurring antibodies also can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Borrebaeck (Ed.), Antibody Engineering (Second edition) New York: Oxford University Press (1995), which is incorporated herein by reference.

An antibody selective for a polypeptide, or that selectively binds a polypeptide, binds with substantially higher affinity to that polypeptide than to an unrelated polypeptide. An antibody selective for a polypeptide also can be selective for a related polypeptide. For example, an antibody selective for

human JAGGED1 (SEQ ID NO:2) also can be selective for hJAGGED2 (SEQ ID NO:4) or for JAGGED1 homologs from other species.

An anti-JAGGED antibody can be prepared, for example, using a JAGGED fusion protein or a synthetic peptide encoding a portion of JAGGED such as hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4) as an immunogen. One skilled in the art would know that a purified JAGGED polypeptide, which can be prepared from natural sources or produced recombinantly as described above, or fragments of JAGGED, including a peptide portion of JAGGED such as a synthetic peptide, can be used as an immunogen. Non-immunogenic fragments or synthetic peptides of JAGGED can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). In addition, various other carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art and described, for example, by Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1988), which is incorporated herein by reference.

The present invention also provides an isolated nucleic acid molecule that contains a nucleotide sequence encoding substantially the same amino acid sequence as JAGGED, or an active fragment thereof, provided that the nucleic acid molecule does not encode the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. An isolated nucleic acid molecule of the invention can have a nucleotide sequence encoding the same amino acid sequence as hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4) or can encode an amino acid sequence with substantial similarity to SEQ ID NO:2 or SEQ ID NO:4, provided that the nucleic acid molecule does not encode the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. An isolated

nucleic acid molecule of the invention can have, for example, a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. Such isolated nucleic acid molecules are exemplified herein as SEQ ID NO:1 and SEQ ID NO:3.

In one embodiment, the invention provides an isolated nucleic acid molecule that contains a nucleotide sequence encoding substantially the same amino acid sequence as JAGGED, or an active fragment thereof, provided that nucleic acid sequence does not encode the amino acid sequence of SEQ ID NO:5, the amino acid sequence of SEQ ID NO:6, the amino acid sequence designated by GenBank accession number U61276, the amino acid sequence designated by GenBank accession number U77720, or the amino acid sequence designated by GenBank accession number U77914.

As used herein, the term "isolated nucleic acid molecule" means a nucleic acid molecule that is in a form that is relatively free from contaminating lipids, polypeptides, unrelated nucleic acids and other cellular material normally associated with a nucleic acid molecule in a cell.

An isolated nucleic acid molecule of the invention can be, for example, a nucleic acid molecule encoding an alternatively spliced JAGGED variant, a polymorphic variant, a nucleic acid molecule that is related, but different, and encodes the same JAGGED polypeptide due to the degeneracy of the genetic code, or a nucleic acid molecule that is related, but different and encodes a different JAGGED polypeptide that exhibits at least one biological activity of JAGGED, provided that the nucleic acid molecule does not encode the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6.

The present invention also provides a cell containing a recombinant nucleic acid molecule having a nucleotide sequence encoding substantially the same amino acid as JAGGED, or active fragment thereof, provided that the nucleotide sequence does not encode the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. The encoded JAGGED polypeptide can be, for example, hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4), or an active fragment thereof, including soluble active fragments and membrane-bound active fragments. The cell can be a prokaryotic cell or a eukaryotic cell such as an HS-23 human stromal cell, COS cell or BHK cell.

An HS-23 cell can be particularly useful for expressing a recombinant nucleic acid molecule encoding a membrane-bound form of a JAGGED polypeptide. HS-23 cells can be transduced with retroviral vectors to express membrane-bound JAGGED variants and can be used as a stromal cell layer for maintaining hematopoietic progenitor cells and inhibiting their differentiation. As described in Example II, a COS or BHK cell can be particularly useful for expressing a recombinant nucleic acid molecule encoding a soluble form of JAGGED, such as an active fragment having hJAGGED1 amino acids 1 to 1010 (SEQ ID NO:7) or an active fragment having hJAGGED1 amino acids 178 to 240 (SEQ ID NO:8). The supernatant from such a COS or BHK cell has the activity of the soluble active JAGGED fragment and can be used in crude form to inhibit the differentiation of hematopoietic progenitor cells or as a source for purifying the soluble active JAGGED fragment.

The present invention also provides an isolated JAGGED peptide having at most about 40 amino acids and including substantially the same amino acid sequence as SEQ ID NO:9. A JAGGED peptide of the invention can be, for example, a peptide of up to about forty amino acids

including the amino acid sequence SEQ ID NO:9, or a substantially similar sequence. A JAGGED peptide can have, for example, about 20, 25, 30, 35 or 40 amino acids including the amino acid sequence of SEQ ID NO:9 or a substantially similar sequence. Provided herein is an example of an isolated JAGGED peptide, which has the amino acid sequence Cys-Asp-Asp-Tyr-Tyr-Tyr-Gly-Phe-Gly-Cys-Asn-Lys-Phe-Cys-Arg-Pro-Arg (SEQ ID NO:9).

The JAGGED peptide SEQ ID NO:9 has the amino acid sequence of residues 188 to 204 of hJAGGED1, which corresponds to a portion of the conserved DSL domain. As disclosed herein, this 17-mer peptide SEQ ID NO:9 can mimic the function of hJAGGED1 in promoting survival and inhibiting differentiation of Notch-expressing myeloid progenitor cells in the presence of a differentiating stimulus. Figure 4 shows that differentiation of 32D clones expressing Notch1 was unaffected by treatment with peptide SEQ ID NO:10 ("J-B") or SEQ ID NO:11 ("J-C"). However, differentiation was significantly inhibited in the presence of the JAGGED peptide SEQ ID NO:9 ("J-A") as shown in the lower right panel of Figure 4. This inhibition was similar to that observed when Notch-expressing 32D cells were cultured with hJAGGED1-expressing HS-27a stromal cells. Thus, a JAGGED peptide of the invention has activity in inhibiting the differentiation of progenitor cells and can be useful, for example, in the *in vitro* expansion of a variety of hematopoietic progenitor cell types.

The present invention therefore provides methods of using the JAGGED polypeptides and peptides of the invention. The present invention provides a method of inhibiting differentiation of hematopoietic progenitor cells by contacting the hematopoietic progenitor cells with an isolated JAGGED polypeptide having substantially the same amino acid sequence as JAGGED, or an active

fragment thereof. An isolated JAGGED polypeptide useful in the methods of the invention can have substantially the same amino acid sequence as hJAGGED1 (SEQ ID NO:2) or hJAGGED2 (SEQ ID NO:4) or can be an active fragment.

The invention also provides a method of inhibiting differentiation of progenitor cells by contacting the progenitor cells with an isolated JAGGED peptide having at most about forty amino acids and including substantially the same amino acid sequence as SEQ ID NO:9. Such progenitor cells can be hematopoietic progenitor cells and can be contacted, for example, *in vitro*. Such an isolated JAGGED peptide of the invention can be, for example, a peptide of up to about forty amino acids which includes the amino acid sequence SEQ ID NO:9 or a substantially similar sequence. For example, an isolated JAGGED peptide useful in the methods of the invention can be a peptide having the sequence Cys-Asp-Asp-Tyr-Tyr-Tyr-Gly-Phe-Gly-Cys-Asn-Lys-Phe-Cys-Arg-Pro-Arg (SEQ ID NO:9).

As used herein, the term "progenitor cell" means any cell capable of both self-renewal and differentiation. Thus, a progenitor cell can proliferate under appropriate conditions to produce an increased number of progenitor cells, or can differentiate under appropriate conditions to produce cells of specialized function. A progenitor cell can be a committed or unipotent progenitor cell that differentiates into one particular differentiated cell type. A progenitor cell also can be a pluripotent progenitor cell that has the potential to differentiate into multiple different cell types. A progenitor cell can be, for example, a hematopoietic progenitor cell, a neuronal precursor cell, a muscle progenitor cell, a hepatic progenitor cell or another cell capable of both self-renewal and differentiation. One skilled in the art understands that

a progenitor cell useful in the invention expresses a JAGGED receptor, which can be, for example, a Notch polypeptide.

The term "hematopoietic progenitor cell," as used herein, means a progenitor cell capable of differentiating to one or more red or white blood cell types. A hematopoietic progenitor cell can be, for example, a totipotent hematopoietic stem cell capable of both self-renewing and differentiating to all hematopoietic cell types, thereby producing erythrocytes, granulocytes, monocytes, mast cells, lymphocytes and megakaryocytes. A hematopoietic progenitor cell also can be, for example, a lymphoid progenitor or myeloid progenitor cell. A lymphoid progenitor cell generates T and B progenitor lymphocytes. A myeloid progenitor cell generates progenitor cells for erythrocytes, neutrophils, eosinophils, basophils, monocytes, mast cells and platelets. In nature, bone marrow stromal cells produce the membrane-bound and diffusible factors responsible for maintaining an appropriate balance between hematopoietic progenitor cell proliferation and differentiation.

The present invention provides methods of maintaining progenitor cells in an undifferentiated state by contacting progenitor cells with a JAGGED polypeptide, or active fragment thereof. The progenitor cells can be cells capable of reconstituting the hematopoietic system such as hematopoietic stem cells. In one embodiment, the progenitor cells are maintained in a totipotent state capable of differentiating into all the specialized cell types of the hematopoietic system.

Subsequent to treating progenitor cells according to a method of the invention, the progenitor cells can be subject to cryopreservation, for example, by freezing in liquid nitrogen and can be stored, if

desired, for a period of months, years or decades and later thawed for further expansion or differentiation. Thus, progenitor cells can be obtained from a newborn, for example, "locked" into an undifferentiated state using a JAGGED polypeptide according to a method of the invention, and stored for future use for an indefinite period.

The methods of the invention also represent advances in cell transplantation and gene therapy. In one embodiment, progenitor cells maintained in an undifferentiated state according to the methods of the invention can be subsequently transplanted into an individual, such that the progenitor cells differentiate fully in the individual. The progenitor cells can be, for example, totipotent hematopoietic stem cells, which differentiate fully in the individual to reconstitute the hematopoietic system.

The methods of the invention therefore have utility in cell transplantation, including bone marrow transplantation, peripheral blood stem cell transplantation and umbilical cord blood transplantation (McAdams et al., Trends in Biotech. 14:341-349 (1996), which is incorporated herein by reference). The cell transplantation methods of the invention can be useful, for example, in replacing the hematopoietic stem cells of a cancer patient having a leukemia or lymphoma such as acute myelogeous leukemia (AML), non-Hodgkin's lymphoma or chronic myelogenous leukemia.

The progenitor cells can be, for example, autologous or allogeneic to the individual into which the transplanted cells are introduced. When the progenitor cells are derived from a cancer patient, the progenitor cells can be obtained by purging bone marrow or peripheral blood with, for example, chemical agents,

immunomagnetic beads, antisense oligonucleotides or antibodies. If desired, progenitor cells can be sorted prior to treating with a JAGGED polypeptide, or active fragment thereof, according to a method of the invention. For example, progenitor cells can be sorted to obtain $CD34^+$ stem cells, which are contacted with a JAGGED polypeptide or active fragment thereof to maintain the $CD34^+$ stem cells in an undifferentiated state capable of full differentiation, and subsequently transplanted into an individual such that the $CD34^+$ stem cells differentiate fully and reconstitute the entire hematopoietic system of the individual.

The methods of the invention also have gene therapy applications. A nucleic acid molecule encoding a gene product can be introduced into progenitor cells maintained in an undifferentiated state according to a method of the invention. Gene therapy methods for introducing a nucleic acid molecule into a cell such as a progenitor cell are well known in the art and include retroviral and adenoviral methods as well as liposome-mediated and other gene transfer technologies as described in Chang (Ed), Somatic Gene Therapy Boca Raton, CRC Press, Inc. (1995), which is incorporated herein by reference. The methods of the invention, involving the use of a JAGGED polypeptide or JAGGED peptide for maintaining progenitor cells in an undifferentiated state, are particularly useful when combined with retroviral gene transfer methods, which require that cells be in a proliferating state.

The invention also provides a method of maintaining progenitor cells in an undifferentiated state by contacting the progenitor cells with a JAGGED peptide having at most about 40 amino acids and containing substantially the same amino acid sequence as SEQ ID NO:9. In the methods of the invention, the progenitor

cells can be capable of reconstituting the hematopoietic system. The progenitor cells can be maintained in a totipotent state and can be, for example, maintained in culture.

The invention further provides a method of maintaining progenitor cells in an undifferentiated state by contacting the progenitor cells with a JAGGED peptide having at most about 40 amino acids and containing substantially the same amino acid sequence as SEQ ID NO:9 and cryopreserving the progenitor cells maintained in an undifferentiated state. In addition, the invention provides a method of maintaining progenitor cells in an undifferentiated state by contacting the progenitor cells with a JAGGED peptide having at most about 40 amino acids and containing substantially the same amino acid sequence as SEQ ID NO:9 and introducing a nucleic acid molecule encoding a gene product into the progenitor cells.

The JAGGED polypeptides, active fragments and JAGGED peptides of the invention can be administered in a variety of dosage regimes to modulate the inhibitory effect on undifferentiated hematopoietic progenitor cells. For example, a JAGGED polypeptide, active fragment or JAGGED peptide can be administered in a single bolus of an effective concentration, or alternatively, multiple treatments of a JAGGED polypeptide, active fragment or JAGGED peptide can be administered to, for example, modulate or enhance the inhibitory effect on hematopoietic progenitor cells. Similarly, the amount of a JAGGED polypeptide, active fragment or JAGGED peptide that is administered can be increased or decreased so as to modulate the inhibitory effect on hematopoietic progenitor cell differentiation. A JAGGED polypeptide, active fragment or JAGGED peptide also can be administered in combination with other compounds which can modulate hematopoietic cell

differentiation or can modulate other therapeutic events. Such procedures are known to those skilled in the art.

The inhibition of hematopoietic progenitor cell differentiation also can be modulated by altering the activity of a JAGGED polypeptide receptor. Activity can be altered by, for example, increasing the amount or expression level of a JAGGED polypeptide or by modulating the activation of a JAGGED receptor. Other methods exist as well and are known or can be determined by those skilled in the art.

As disclosed herein, molecular defects in hJAGGED1 can cause Alagille Syndrome, which is an autosomal dominant, developmental disorder that affects structures in the liver, heart, skeleton, eye, face, kidney and other organs. The minimal estimated frequency of the syndrome is 1 in 70,000 live births. The syndrome traditionally has been defined by a paucity of intrahepatic bile ducts in association with several of the main clinical abnormalities: cholestasis, cardiac disease, skeletal abnormalities, ocular abnormalities and a characteristic facial phenotype. Cholestasis occurs as a consequence of the paucity of bile ducts. Cardiac anomalies most commonly involve the peripheral and main pulmonary arteries as well as the pulmonary valves. The most common skeletal anomalies are "butterfly" or hemivertebrae, resulting from clefting abnormalities of the vertebral bodies. Ocular lesions include anterior chamber defects, most commonly posterior embryotoxon, which is a benign defect, and retinal pigmentary abnormalities. Facies have been described as triangular, consisting of a prominent forehead, deep-set eyes, hypertelorism, long straight nose with flattened tip, short philtrum, flat midface and a triangular chin. Renal and neurodevelopmental abnormalities occur less frequently. Fifteen percent of patients will require

liver transplantation and seven to ten percent of patients have severe congenital heart disease, most often tetralogy of Fallot (Walker et al. (Eds), Gastrointestinal Disease: Pathophysiology, Diagnosis, Management (3rd edition) B.C. Decker, Inc., Philadelphia pp 1124-1140 (1991), which is incorporated herein by reference). An Alagille Syndrome diagnosis is made if bile duct paucity is accompanied by three of the five main clinical criteria. The expressivity of Alagille Syndrome is variable; accordingly, family members of a proband are considered affected if they express any of the five main clinical features.

The genetic defect underlying this multi-system disorder has been mapped to a 1.5 Mb segment based on analysis of overlapping chromosomal deletions at 20p11-12. Identified herein is the gene responsible for the Alagille Syndrome disorder, the human Notch ligand, hJAGGED1. Four distinct coding region mutations in the hJAGGED1 gene were identified and shown to segregate with disease phenotype in four Alagille Syndrome families. As disclosed in Example V and summarized in Figure 9, all four mutations lie within conserved regions of the hJAGGED1 gene: within the DSL domain, the EGF-repeats and the cysteine-rich region. Each of these mutations are predicted to produce a translational frameshift resulting in a gross alteration of the hJAGGED1 gene product. Furthermore, none of the mutations observed in Alagille Syndrome families were present in 100 normal control chromosomes studied. Thus, from the hundreds of potential genes within the cytogenetic deletion 20p11-12, the hJAGGED1 gene product has been identified as responsible for Alagille Syndrome. Based on this identification, the present invention provides methods of diagnosing Alagille Syndrome in a individual. Such methods can be useful in the early diagnosis or prenatal testing of individuals at risk for the disorder and can

facilitate the development of therapies for affected individuals.

The present invention provides a method of diagnosing Alagille Syndrome in an individual by detecting a disease-associated mutation linked to a JAGGED locus. The disease-associated mutation can be linked but outside a JAGGED gene or can be within a JAGGED gene, for example, in a JAGGED coding sequence, 5' or 3' regulatory region, or within an intronic sequence.

In one embodiment of the invention, the JAGGED locus is a human JAGGED1 (hJAGGED1) locus. In the methods of the invention, the disease-associated mutation can produce, for example, an inactive hJAGGED1 gene product such as a truncated hJAGGED1 gene product. Examples of Alagille Syndrome disease-associated mutations occurring within the hJAGGED1 nucleotide sequence SEQ ID NO:1 are provided herein and include nucleotide variations at nucleotides 1104-1105, nucleotide 3102, nucleotides 2531-2534 and nucleotide 2066 of SEQ ID NO:1.

As used herein, the term "linked" means that two genetic loci have a tendency to be inherited together as a result of their proximity. If two genetic loci are linked and are polymorphic, one locus can serve as a marker for the inheritance of the second locus. Thus, an Alagille Syndrome disease-associated mutation linked to a JAGGED locus having a modified JAGGED allele causing Alagille Syndrome can serve as a marker for inheritance of the modified JAGGED allele. Such a linked mutation can be located in proximity to a JAGGED gene or can be located within a JAGGED gene.

The term "JAGGED locus," as used herein, means a locus encoding a JAGGED gene product. A JAGGED locus can be, for example, the human JAGGED1 locus, positioned within markers D20S894 and D20S507, as described in Example III.

The term "Alagille Syndrome disease-associated mutation," as used herein, is synonymous with "disease-associated mutation" and means a molecular variation of at most several thousand nucleotides that tends to be inherited together with the Alagille Syndrome disorder.

Disclosed herein are a variety of Alagille Syndrome disease-associated mutations linked to the hJAGGED1 locus. Distinct disease-associated mutations, which occur within the hJAGGED1 coding sequence, were found in each of four Alagille Syndrome families as summarized in Figure 9. In a first Alagille Syndrome family, a deletion of "AG" at positions 1104-1105 of SEQ ID NO:1 produced a protein truncated at amino acid 240. In a second family, an insertion of five nucleotides ("GTGGC") at position 3102 of SEQ ID NO:1 produced a protein truncated at amino acid 945, while in a third family, a deletion of "CAGT" at positions 2531-2534 of SEQ ID NO:1 resulted in a protein truncated at amino acid 741. In a fourth Alagille Syndrome family, a single "C" nucleotide deletion at position 2066 of SEQ ID NO:1 resulted in a protein truncated at amino acid 563.

A disease-associated mutation useful in diagnosing Alagille Syndrome can be, for example, a nucleotide substitution, insertion or deletion of one or more nucleotides that tends to be inherited together with Alagille Syndrome. For example, the molecular variation can be a nucleotide substitution, insertion or deletion of about 1 to 3000 nucleotides, such as a substitution,

insertion or deletion of about 1 to 1000 nucleotides, about 1 to 100 nucleotides, about 1 to 50 nucleotides or about 1 to 10 nucleotides. Disclosed herein are a two nucleotide deletion, five nucleotide insertion, four nucleotide deletion and single nucleotide deletion, which are mutations associated with Alagille Syndrome (Example V). One skilled in the art understands that a disease-associated mutation also can be a molecular variation such as abnormal methylation or other modification that does not produce a difference in the primary nucleotide sequence of the disease-associated allele as compared to the normal allele. Specifically excluded from the definition of an Alagille Syndrome disease-associated mutation are large nucleotide variations of more than several thousand nucleotides, including gross cytogenetic deletions and megabase deletions such as those reported in Rand et al., Am. J. Hum. Genet. 57:1068-1073 (1995), which is incorporated herein by reference.

An Alagille Syndrome disease-associated mutation can occur within a JAGGED gene and can result, for example, in production of an inactive JAGGED gene product or a reduced amount of a JAGGED gene product. For example, an Alagille Syndrome disease-associated mutation within a JAGGED gene can be a nucleotide modification within a gene regulatory element such that a JAGGED gene product is not produced or a nucleotide modification within an intronic sequence resulting in an abnormally spliced, inactive JAGGED gene product. In addition, an Alagille Syndrome disease-associated polymorphism can be a nucleotide modification resulting in one or more amino acid substitutions, deletions or insertions in a JAGGED coding sequence, which result in an inactive JAGGED gene product. For example, an inactive JAGGED gene product can result from a frameshift or nonsense mutation producing a truncated JAGGED gene

product, a missense mutation, or a gross nucleotide insertion or deletion. Such an inactive JAGGED gene product can be, for example, a JAGGED polypeptide variant lacking the ability to activate Notch or a soluble JAGGED polypeptide that functions as a dominant negative molecule when expressed with wild type JAGGED polypeptide or another JAGGED polypeptide variant lacking one or more biological functions of JAGGED.

A variety of molecular methods useful in detecting an Alagille Syndrome disease-associated mutation linked to a JAGGED locus are well known in the art. For example, allele-specific oligonucleotide hybridization involves the use of a labeled oligonucleotide probe having a sequence perfectly complementary, for example, to a disease-associated sequence. Under appropriate conditions, the allele-specific probe hybridizes to a nucleic acid containing the disease-associated mutation but does not hybridize to the corresponding wild type nucleic acid sequence having one or more nucleotide mismatches. If desired, a second allele-specific oligonucleotide probe that matches the wild type sequence also can be used. Similarly, the technique of allele-specific oligonucleotide amplification can be used to selectively amplify, for example, a disease-associated polymorphic sequence by using an allele-specific oligonucleotide primer that is perfectly complementary to the nucleotide sequence of a disease-associated allele but which has one or more mismatches as compared to the corresponding wild type sequence (Mullis et al. (Eds.), The Polymerase Chain Reaction, Birkhäuser, Boston, (1994), which is incorporated herein by reference). Particularly useful allele-specific oligonucleotides are oligonucleotides that correspond to about 15 to about 40 nucleotides of the hJAGGED1 nucleotide sequence SEQ ID NO:1 and that include one of the disease-associated polymorphic regions

identified herein: nucleotides 1104-1105, nucleotide 3102, nucleotides 2531-2534 or nucleotide 2066 of SEQ ID NO:1. One skilled in the art understands that the one or more nucleotide mismatches that distinguish between the disease-associated and wild type allele are preferably located in the center of an allele-specific oligonucleotide primer to be used in allele-specific oligonucleotide hybridization. In contrast, an allele-specific oligonucleotide primer to be used in PCR amplification preferably contains the one or more nucleotide mismatches that distinguish between the disease-associated and wild type alleles at the 3' end of the primer.

A heteroduplex mobility assay (HMA) is another well known assay that can be used to diagnose Alagille Syndrome according to a method of the invention. HMA is useful for detecting the presence of a polymorphic sequence since a DNA duplex carrying a mismatch, such as a heteroduplex between a wild type and mutated DNA fragment, has reduced mobility in a polyacrylamide gel compared to the mobility of a perfectly base-paired duplex (Delwart et al., Science 262:1257-1261 (1993); White et al., Genomics 12:301-306 (1992), each of which is incorporated herein by reference). Methods for detecting an Alagille Syndrome disease-associated mutation using a heteroduplex mobility assay are set forth in Example V.

The technique of single strand conformation polymorphism (SSCP) also can be used to detect the presence of an Alagille Syndrome disease-associated mutation (see Hayashi, PCR Methods Applic. 1:34-38 (1991), which is incorporated herein by reference). This technique can be used to detect mutations based on differences in the secondary structure of single-strand DNA that produce an altered electrophoretic mobility upon

non-denaturing gel electrophoresis. Polymorphic fragments are detected by comparison of the electrophoretic pattern of the test fragment to the corresponding fragment from a normal individual of a non-Alagille Syndrome family. The detection of an Alagille Syndrome disease-associated mutation using SSCP is exemplified in Example V.

Denaturing gradient gel electrophoresis (DGGE) also can be used to detect an Alagille Syndrome disease-associated mutation linked to a JAGGED locus. In DGGE, double-stranded DNA is electrophoresed in a gel containing an increasing concentration of denaturant; double-stranded fragments made up of mismatched wild type and disease-associated sequences have segments that melt more rapidly, causing such fragments to migrate differently as compared to perfectly complementary sequences obtained from normal individuals (Sheffield et al., "Identifying DNA Polymorphisms by Denaturing Gradient Gel Electrophoresis" in Innis et al., *supra*, 1990).

Other well-known approaches for analyzing a mutation include automated sequencing, RNAase mismatch techniques (Winter et al., Proc. Natl. Acad. Sci. 82:7575-7579 (1985), which is incorporated herein by reference) and the use of restriction fragment length polymorphisms (see Innis et al., *supra*, 1990). For families in which the disease-associated mutation has been defined, automated sequencing of the region of interest can be particularly useful in diagnosing Alagille Syndrome. Thus, the methods of the invention for diagnosing Alagille Syndrome in an individual can be practiced using a heteroduplex mobility assay or single strand conformation polymorphism assay as illustrated in Example V, using one of the well known assays described

above, or another art-recognized assay for detecting a disease-associated mutation.

The present invention also relates to the presence of genetic polymorphisms in human JAGGED2 and their association with a human syndrome characterized by syndactyly and cleft palate or lip. As disclosed herein, the hJAGGED2 gene can be responsible for the developmental abnormalities in patients with syndactyly, with cleft palate or lip, or with both syndactyly and cleft palate or lip.

Thus, the present invention provides a method of diagnosing a syndrome characterized by syndactyly and cleft palate or lip in a human, comprising detecting a syndactyly and cleft palate or lip-associated mutation linked to a human JAGGED2 locus. In such a method, the syndrome-associated mutation can be within a hJAGGED2 locus, for example, within a hJAGGED2 regulatory element or coding sequence. A syndrome associated mutation can produce, for example, a point mutation or truncation that alters the expression or activity of hJAGGED2.

A mutation associated with a syndrome characterized by syndactyly and cleft palate or lip can be detected by a variety of methodologies including, for example, allele-specific oligonucleotide hybridization, denaturing gradient gel electrophoresis, heteroduplex mobility assays, single strand conformation polymorphism assays, automated sequencing, RNAase mismatch techniques, or restriction fragment length polymorphism-based approaches, as described above in regard to the detection of mutations associated with Alagille Syndrome. The skilled person will recognize that a syndactyly and cleft palate or lip-associated mutation can be detected with these or other routine methodologies known in the art of genetics.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

ISOLATION AND CHARACTERIZATION OF HUMAN JAGGED1

This example describes the isolation, characterization and expression of human JAGGED1.

Isolation of the Human JAGGED1 cDNA

A cDNA encoding a human Notch ligand expressed in the bone marrow microenvironment was isolated by amplifying human bone marrow cDNA with degenerate primers SEQ ID NO:12 and SEQ ID NO:13, which correspond to portions of the conserved DSL and EGF-like repeat domains of rat Jagged1 (rJagged; Lindsell et al., *supra*, 1995). Ten PCR products of potential interest were identified, cloned and sequenced. The clone Sdi-06 contains a 327 bp insert that encodes part of the DSL and EGF-repeat domains. The sequence of this fragment has 96% predicted amino acid sequence identity with the corresponding region of rJagged1 (residues 205 to 312), 84% predicted amino acid sequence identity with C-Serrate-1 (residues 178-286), and 52% predicted amino acid sequence identity with C-Delta-1 (residues 203-311). Thus, the Sdi-06 clone encodes a partial cDNA fragment of the human homolog of rJagged1.

The complete hJAGGED1 cDNA was obtained by screening a human bone marrow cDNA library with ^{32}P-labeled Sdi-06. One of the cDNA clones isolated, D-01, was found to contain the 5'-end of human JAGGED1 including 417 bp of 5' untranslated sequence and 2270 bp of coding sequence. The 3' end of hJAGGED1 was obtained

by rescreening the same human bone marrow cDNA library with ^{32}P-labeled rat Jagged1 cDNA provided by Dr. Weinmaster (Lindsell et al., *supra*, 1995). A cDNA clone identified with this probe, designated Y-A01, contains 2.4 kb of coding region and 1.5 kb of 3' untranslated region. A full-length 5.5 kb hJAGGED1 cDNA was assembled from the 5' D-01 clone and the 3' Y-A01 clone as described further below.

The full-length hJAGGED1 clone has an open reading frame of 3657 base pairs and encodes a predicted protein product of 1219 amino acids (Figure 1A). Analysis of the amino acid sequence indicates that hJAGGED1 is a transmembrane protein with a large extracellular domain and a very short intracellular domain. The hJAGGED1 protein shares structural features with the *Drosophila* Notch ligands Delta and Serrate and with rat Jagged1. The shared structural features include a DSL motif and 16 epidermal growth factor-like (EGF-like) repeats within the extracellular domain. A cysteine-rich region present in Serrate and rJagged1 is also conserved in hJAGGED1 (Figure 1C).

An alignment of the amino acid sequences of hJAGGED1 (hjg) and rJagged1 (rjg) is shown in Figure 2A. The hJAGGED1 protein has 94% overall amino acid identity with rJagged1, with 96% amino acid identity with the highly conserved DSL and EGF-repeat domains. Several distinctive amino acid substitutions are present in the hJAGGED1 sequence relative to rJagged1. Two prolines in the signal peptide region of rJagged1 are replaced with arginine and serine in hJAGGED1 (residues five and ten, respectively). In addition, the region between the signal peptide and the DSL motif is dissimilar (compare residues 56 to 64 in hJAGGED1 (GGARNPGDR; SEQ ID NO:14) to residues 56 to 65 in rJagged1 (AEPGTLVRPY; SEQ ID NO:15). Other amino acid differences include a proline

to phenylalanine substitution within the DSL motif (residue 194 of hJAGGED1); amino acid differences within the EGF-repeat region; and a serine to cysteine substitution within the cysteine-rich domain, (residue 860 of hJAGGED1). In the intracellular domain, a proline to serine substitution occurs at residue 1107 of hJAGGED1, and a valine to proline substitution occurs at residue 1187 of hJAGGED1.

Human bone marrow poly(A) RNA was obtained from Clontech Laboratories, Inc. (Palo Alto, CA) and reverse transcribed with random primer using the SuperScript Preamplication system (catalogue number 18089-011 from Gibco BRL (Gaithersburg, MD) following the manufacturer's procedure. First strand cDNA was subsequently amplified by PCR using degenerate primers SEQ ID NO:12 and SEQ ID NO:13, which correspond to peptide sequences DDFFGHY (residues 205-211; SEQ ID NO:16) and PCHYGGTCRDLVND (residues 676-689; SEQ ID NO:17), respectively. The sequence of SEQ ID NO:12 is 5'-GAYGAYTTYTTYGGNCAYTA-3', and the sequence of SEQ ID NO:13 is 5'-RCANGTNCCNCCRTARTGRCANGG-3', where R indicates G/C, Y indicates T/A, and N indicates G/C/T/A. PCR reactions were performed using Taq polymerase (Perkin Elmer, Foster City, CA) under the following conditions: 92°C, 30 seconds; 50°C, 30 seconds; and 72°C for 1 minute for 35 cycles. Ten candidate PCR products were obtained and cloned into the TA-cloning vector, pCR2l (Invitrogen, San Diego, CA). DNA sequencing was performed using the dyeprimer method with both M13 reverse and -21M13 primers on an ABI automated Sequencer model 377 or 373 (Applied Biosystems, Foster City, California). One of these clones was the 327 bp Sdi-06 clone described above.

To obtain the full-length hJAGGED1 cDNA, a human bone marrow λgt11 cDNA Library (catalogue number HL5005b; Clontech) was screened. The library was plated

at $5x10^4$ pfu on LB/Mg agar according to the manufacturer's protocol. After incubation for 8 to 12 hours, plaques were transferred to nitrocellulose filters (Schleicher & Schuell, Inc., Keene, NH) and denatured, neutralized, and cross-linked by UV irradiation. The filters were prehybridized and hybridized at 60°C with solutions prepared as described in Church and Kieffer-Higgins, Science 240:185-188 (1988), which is incorporated herein by reference. Following hybridization, filters were washed twice with 2XSSC/1%SDS for 10 minutes at room temperature and twice with 0.2XSSC/1%SDS for 20 minutes at 60°C. DNA was isolated from positive clones that were confirmed by a second hybridization under the same conditions. The cDNA clones D-01 and Y-A01, containing the 5' (2.2 kb) and 3' (4.5 kb) cDNA fragments of hJAGGED1, respectively, were cloned into the EcoRI site of the pBluescriptSK-vector (Stratagene, La Jolla, CA).

The full-length hJAGGED1 cDNA (pBS-hJg1) was generated by replacing the 300 bp 5' EcoRI/BglII fragment in Y-A01 with the 1.3 kb 5' EcoRI/BglII cDNA fragment in D-01. The resulting 5.5 kb cDNA clone hJAGGED1 was sequenced using random "shotgun" sequencing essentially as described in Smith et al., Genome Res. 6:1029-1049 (1996), which is incorporated herein by reference. A shotgun library was constructed by sonicating pBS-hJg1 plasmid DNA, size-selecting 1.5-2 kb fragments on an agarose gel, blunting the ends of the size-selected fragments using mung bean nuclease, and cloning the fragments into Sma I-digested M13-mp18 vector (Novagen, Inc., Madison, WI) essentially as described in Rowan and Koop (Eds.), Automated DNA Sequencing and Analysis pp. 167-174, Academic Press, Inc. (1994), and Smith et al., Genome Research 6:1029-1049 (1996), each of which is incorporated herein by reference. Briefly, single-stranded DNA was prepared from single plaques as described in Smith et al., *supra*, 1996. Approximately 80

single-stranded DNA templates were sequenced by ABI thermal-cycle sequencing using fluorescently-labeled -21M13 primer following the manufacturer's procedure. Sequencing data was assembled into a single 5.5 kb contig with approximately 6-fold redundancy using the basecalling and sequence assembly programs Phred and Phrap (P. Green, unpublished, http://www.genome, Washington.edu).

Expression of Human Jagged1 mRNA

In order to evaluate the expression pattern of hJAGGED1, Northern blot analysis was performed on multiple human tissues using a hJAGGED1 fragment as a probe. A single 5.5 kb mRNA transcript was detected in all tissues tested, including stomach, thyroid, spinal cord, lymph node, trachea, adrenal gland, and bone marrow. High levels of hJAGGED1 expression were noted in thyroid and trachea, while relatively lower levels of expression were observed in lymph node and bone marrow. Further Northern analysis demonstrated that hJAGGED1 is also expressed in adult heart, lung, skeletal muscle, kidney and placenta. However, hJAGGED1 expression was undetectable in adult brain or liver tissue.

Analysis of human fetal tissues showed high levels of hJAGGED1 expression in fetal kidney (16-32 weeks) and fetal lung (18-28 weeks), with lower levels of expression in fetal brain (20-25 weeks) and fetal liver (16-32 weeks). Expression of hJAGGED1 in heart, fetal liver, lung and kidney is consistent with a role for the hJAGGED1 protein in the normal development of these tissues.

The results described above demonstrate that hJAGGED1 is expressed in whole bone marrow, a heterogeneous tissue consisting of a variety of stromal

and hematopoietic cell populations. In order to determine whether hJAGGED1 expression is restricted to certain marrow subpopulations, RNA was isolated from primary human bone marrow stromal cells and analyzed by Northern blotting. A 5.5 kb transcript was detected, indicating that hJAGGED1 is expressed in bone marrow stromal cells. Several cell lines representing functionally distinct bone marrow stromal cells also were analyzed for hJAGGED1 expression. These immortalized human bone marrow stromal cell lines, designated HS-5, HS-23, and HS-27a, have been previously characterized (Roecklein and Torok-Storb, Blood 85:997-1005 (1995), which is incorporated herein by reference. The hJAGGED1 transcript was expressed at significant levels in HS-27a cells but was undetectable in HS-5 or HS-23 cells, indicating that hJAGGED1 is differentially expressed in distinct subpopulations of marrow stromal cells.

Northern blot analysis was performed as follows. Northern blots of multiple human tissues and human fetal tissues were obtained from Clontech and probed with ^{32}P-labeled Sdi-06 or a 400 bp fragment of the hJAGGED1 cDNA. The 400 bp probe was prepared by amplification with primer pair 292 (AGATCCTGTCCATGCAGAACGT; SEQ ID NO:18) and 293 (ATACTCAAAGTGGGCAACGCC; SEQ ID NO:19). For analysis of human stromal cells, 10 μg of total RNA was isolated from primary marrow stromal cells or the indicated stromal cell line using Stratagene's mRNA isolation kit (catalogue number 200347). Total RNA was electrophoresed on a formamide denaturing agarose gel and transferred onto Nytran® membrane (Schleicher & Schuell). Membranes were prehybridized and hybridized using Stratagene's QuikHyb® solution at 65°C. ^{32}P-labeled probes were denatured by boiling and added directly to prehybridization solution containing 100 μg salmon sperm DNA per 15 ml solution. Membranes were washed twice in

2X SSC/0.1% SDS at room temperature for 10 minutes, followed by washing once with 0.1X SSC/0.1% SDS at 60°C for 20 minutes. β-Actin cDNA (Clontech) was used as a control for the Northern analysis.

Expression of human JAGGED1 polypeptide

The full-length hJAGGED1 cDNA was cloned into the EcoRI/XhoI sites of the IPTG-inducible prokaryotic expression vector, pET-24b(+) (Novagen). The hJAGGED1 expression vector was transformed into B021(DE3) cells, which are bacterial cells containing the T7 RNA polymerase gene under control of an IPTG-inducible promoter.

A cell extract was prepared from transformed cells induced by 0.1 mM IPTG and from control uninduced cells. The cell extracts were fractionated on SDS-PAGE and transferred to nitrocellulose filters. Western analysis was performed with the ECL system (Amersham, Arlington Heights, IL) using a monoclonal antibody raised against peptide SEQ ID NO:11 ("J-C"), which corresponds to residues 1096 to 1114 of hJAGGED1 (KRRKPGSHTHSASEDNTTN). A polypeptide of about 150 kDa, absent from the control uninduced extract, was detected in the IPTG-induced cell extract. These results indicate that a hJAGGED1 polypeptide can be expressed in bacteria and that bacterially expressed hJAGGED1 exhibits a molecular weight of about 150 kDa.

EXAMPLE II

hJAGGED1 EXPRESSED ON MARROW STROMA INHIBITS HEMATOPOIETIC DIFFERENTIATION

This example demonstrates that a peptide derived from the DSL domain of hJAGGED1 inhibits G-CSF induced granulocytic differentiation of Notch1-expressing myeloid progenitors.

The HS-27a human stromal cell line inhibits differentiation of myeloid progenitors expressing Notch1

The ability of the hJAGGED1 HS-27a human stromal cell line to effect differentiation of hematopoietic progenitors was analyzed using the interleukin-3 (IL-3)-dependent myeloid cell line, 32D. The 32D cell line, which was derived from normal mouse bone marrow, is a heterogeneous cell line with individual cells having characteristics of myeloid cells at various early stages of maturation. 32D cells proliferate as undifferentiated blasts in the presence of IL-3, but differentiate into mature granulocytes when stimulated with granulocyte colony stimulating factor (G-CSF; Valtieri et al. Immunol. 138:3829-3835 (1987), which is incorporated herein by reference), thereby providing a system for analyzing factors that may affect myeloid differentiation.

Expression of an activated form of murine Notch1 inhibits G-CSF-induced granulocytic differentiation of 32D cells while permitting expansion of undifferentiated progenitor cells (Milner et al., *supra*, 1996). The function of hJAGGED1 was assayed by transducing 32D cells with a full-length Notch1 cDNA and evaluating the differentiative capacity of the transduced cells under several culture conditions. As shown in

Figure 3A, 32D clones expressing full-length Notch1 differentiate in response to G-CSF in a manner similar to parental 32D cells (WT) or clones expressing control retroviral constructs (LXSN). In contrast, 32D clones expressing the activated intracellular domain of Notch1 (N1-ICΔOP) remain primarily undifferentiated under these conditions, consistent with the results reported in Milner et al., *supra*, 1996 (Figure 3A).

Full-length Notch1-expressing 32D myeloid progenitors were co-cultured with hJAGGED1-expressing HS-27a human stromal cells, and differentiation of the 32D cells assayed. Figure 3B shows the differentiation patterns of 32D clones expressing full-length Notch1 or the control pLXSN retrovirus in the presence of G-CSF on monolayers of HS-27a, HS-23 or HS-5 stromal cells. LXSN control clones differentiate into mature granulocytes when cultured on any of these cell lines (Figure 3B, left panels); by day 6, 50-80% of the cells have a mature phenotype, and less than 15% remain undifferentiated. Full-length Notch1-expressing 32D cells also differentiate in response to G-CSF when cultured on the HS-23 or HS-5 lines, but granulocytic differentiation is significantly inhibited in the presence of HS-27a cells (Figure 3B, right panels). When cultured on HS-23 or HS-5 cells, 40-50% of the cells are mature with 15-20% remaining undifferentiated by day 6. In contrast, only 20% of the 32D cells are mature with 40% remaining undifferentiated when cultured on the HS-27a stromal cell line. The middle panels of Figure 3B show representative Wright stained cytospins of cells after four days in culture. The greatest difference between control and Notch1-expressing 32D cells occurs in the HS-27a co-cultures. These findings demonstrate that a specific interaction between HS-27a cells and Notch1 on 32D cells inhibits granulocytic differentiation, indicating that

hJAGGED1 is capable of activating Notch1 in myeloid progenitor cells.

The maintenance of undifferentiated progenitor cells was analyzed under different culture conditions by determining the total number of viable cells and the relative percentages of undifferentiated and mature cells remaining in the cultures on consecutive days. As shown in Table 1, cultures of 32D cells expressing full-length Notch1 maintain close to the original number of cells (90%) as undifferentiated progenitors after five days in G-CSF when cultured on HS-27a stromal cells. This result contrasts with cultures of control 32D cells, in which significantly fewer viable cells remain, almost all of which are differentiated. In the control 32D cells, fewer than 5% of the original number of cells are maintained as undifferentiated cells. Cultures of full-length Notch1-expressing 32D cells also had slightly greater numbers of undifferentiated cells remaining after five days when cultured on HS-23 or HS-5 stromal cells compared to cultures of the control 32D cells. However, cultures of full-length Notch1-expressing 32D cells grown on HS-27a contained significantly greater numbers of undifferentiated cells than those grown on either HS-23 or HS-5.

Table 1

Maintenance of undifferentiated cells after culture in the presence of G-CSF and stromal cell lines.

	Percent of original number of cells plated remaining undifferentiated			Replating efficiency
32D Clone	HS-27a	HS-23	HS-5	HS-27a
LXSN	5±4.7	4±3	2±1.2	11%
FL Notch1	90±28	15±2.6	19±29	190%

To verify that cells appearing undifferentiated by morphology were both viable and capable of continued proliferation as undifferentiated cells, cells were replated in WEH1 conditioned media (WCM) containing IL-3 after 6 days in culture with G-CSF and HS-27a cells. The cloning efficiency was evaluated by serial dilutions in 96-well plates as described further below. Compared to the original number of cells plated, the calculated percentage of clonable cells remaining was 190% for the full-length Notch1-expressing 32D cells and 11% for control 32D cells (see Table 1). These results indicate that co-culture of Notch1-expressing 32D cells in the presence of hJAGGED1-expressing HS-27a cells permits survival and maintains the proliferative potential of undifferentiated myeloid cells even in the presence of a differentiative stimulus such as G-CSF.

Notch1 cDNA retroviral vectors were constructed and transduced as follows. The full length clone of murine Notch1, provided by Drs. Jeff Nye and Raphael Kopan (Nye et al., Development 120:2421-2430 (1994); and Kopan and Weintraub, J. Cell Biol. 121:631-641 (1993), each of which is incorporated herein by reference) was subcloned into the EcoRI site of the pLXSN retroviral vector (Milner et al., *supra*, 1996). Retroviral producer cell lines expressing Notch1 were generated essentially as described in Milner et al., *supra*, 1996, and construct expression was confirmed by RT-PCR or western blot analysis. 32D cells were transduced by transwell co-cultivation with Notch1/PA317 producer cells as described in Milner et al., *supra*, 1996.

Notch1-expressing 32D clones were selected in G418 and expanded, and expression was confirmed by RT-PCR and western blotting using a monoclonal antibody generated against the intracellular domain of murine Notch1 provided by L. Milner.

The HS-27a, HS-23 and HS-5 human stromal cell lines were maintained in RPMI containing 10% FCS as described in Roecklein and Torok-Storb, *supra*, 1995. 32D cells were maintained in Iscove's Modified Dulbecco's Medium (IMDM) with 10% fetal bovine serum (FBS) and 10% WCM as a source of IL-3. For differentiation experiments, 32D cell lines were harvested in log phase, washed, counted, and replated at constant density ($2x10^5$ cells/ml, 4 ml/well) in 6-well plates in IMDM, 10% FBS, 0.5% WCM and 20 ng/ml recombinant human G-CSF from Amgen (Thousand Oaks, CA). Aliquots of 20 ml were removed daily for analysis and replaced with fresh media. Viable cells were counted, and Wright stained cytospins were evaluated for granulocytic differentiation as follows. Undifferentiated 32D cells generally had a single large, relatively round nucleus and scant dark blue cytoplasm containing few large granules. Criteria for granulocytic differentiation included nuclear segmentation, an increased cytoplasmic to nuclear ratio, and increased eosinophilia and granularity of the cytoplasm. Differential cell counts were performed on 100-200 cells on several occasions and in random/blinded fashion by the same individual (LM) to ensure consistency. The differential cell counts were confirmed by independent observers in a blinded fashion.

For co-culture experiments with 32D cells, human stromal cell lines were cultured in 6-well plates to approximately 75% confluence, washed and plated with 32D cells as described above, with the exception that 32D cells were plated at a density of $4x10^5$ cells/ml in 2 ml on the stromal cell layer and incubated for one to two hours prior to the addition of media containing G-CSF.

For assessment of cloning efficiency shown in Table 1, 32D cells were cultured at various cell densities ($2X10^5$, $1X10^4$, or $2.5X10^4$/ml) in 6-well plates

as described above. After 6 days in culture with media containing 20 ng/ml G-CSF and 0.5% WCM on HS-27a stromal layers, 32D cells were harvested and replated in triplicate in 10% WCM in 96-well plates. Serial dilutions were made, and wells assessed for growth daily for seven to ten days. Positive wells all showed continued proliferation during the period of observation and contained greater than 100 cells by day seven to ten.

A hJAGGED1 DSL peptide inhibits differentiation of Notch1-expressing myeloid progenitors

Three peptides corresponding to different regions of the hJAGGED1 molecule were analyzed for their effect on differentiation of Notch1-expressing 32D cells treated with G-CSF. Peptide SEQ ID NO:9 ("J-A") contains residues 188 to 204 of hJAGGED1 and corresponds to a hydrophilic portion of the conserved DSL domain, which is a domain unique to putative Notch ligands. Peptide SEQ ID NO:10 ("J-B") contains residues 235 to 257 and corresponds to part of EGF-repeat 1 in the extracellular domain. Peptide SEQ ID NO:11 ("J-C") contains residues 1096 to 1114 and corresponds to a hydrophilic portion of the intracellular domain. Figure 4 shows differentiation of control (LXSN) and full-length Notch1-expressing 32D cells in response to G-CSF in the presence of peptide SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:11. G-CSF-induced differentiation of control clones was unchanged by the addition of any of the peptides (Figure 4, left panels; compare to G-CSF alone in Figure 3A). Differentiation of the full-length Notch1-expressing 32D clones in the presence of G-CSF and either peptide SEQ ID NO:10 or SEQ ID NO:11 ("J-B" or "J-C"; Figure 4, top right) was comparable to that observed with G-CSF alone (see Figure 3A). In contrast, differentiation was significantly inhibited in the presence of peptide SEQ ID NO:9 ("J-A") (Figure 4, lower right). The extent of

inhibition was similar to that observed when these cells were co-cultured on the HS-27a monolayer in the presence of G-CSF (see Figure 3B).

Peptide SEQ ID NO:9 ("J-A") has the sequence CDDYYYGFGCNKFCRPR. Peptide SEQ ID NO:10 ("J-B") has the sequence CRQGCSPKHGSCKLPGDCRCQYG); and peptide SEQ ID NO:11 ("J-C") has the sequence KRRKPGSHTHSASEDNTTN. Each of these peptides were synthesized at the University of Washington Biopolymer Facility. Differentiation of 32D cells in the presence of hJAGGED peptides was analyzed as described above. 32D cells were incubated in media containing 20 μM peptide for 1 hour prior to the addition of G-CSF to a final concentration of 20 ng/ml. The final peptide concentration for the experiment depicted in Figure 4 was 10 μM. Fresh peptide was added to the original concentration on day 4 of culture.

An active fragment of hJAGGED1 inhibits granulocytic differentiation of mouse hematopoietic progenitor cells

A soluble fragment of hJAGGED1 (SEQ ID NO:7), which contains the extracellular domain of hJAGGED1 including the signal peptide, DSL region, EGF-like repeats and cysteine-rich region, was prepared by amplifying a portion of the hJAGGED1 cDNA with PCR primers 420 (SEQ ID NO:20; CCGCTCGAGACCATGCGTTCCCCACGGA) and 421 (SEQ ID NO:21; CGGAATTCTCAGTGGTGGTGGTGGTGTTCATTGTTCGCTGAA). The hJAGGED1 cDNA fragment, corresponding to residues 1 to 1010, was subcloned into expression vector pDX to generate pDX-hJg1.Ex. After transfection into BHK and COS cells, the cell culture supernatant was assayed for the ability to effect the number of G-CFU formed from mouse hematopoietic progenitor cells (Sca-1$^+$ lin$^-$), which were prepared by removing cells that stained with anti-Gr-1, anti-CD4, anti-CD11b, anti-CD2, anti-CD45R and

anti-Ter-119 and then positively selecting Sca-1$^+$ cells with anti-Sca-1. As shown in Table 2, supernatant from BHK cells transfected with the hJAGGED1 extracellular domain construct reduced the average number of colony forming units (CFU-G-CSF) of Sca-1$^+$ lin$^-$ cells treated with G-CSF from about 60 to about 24. These results indicate that the hJAGGED1 fragment SEQ ID NO:7 encoding the extracellular domain of hJAGGED1 (residues 1 to 1010) inhibits granulocytic differentiation and is an active fragment of hJAGGED1.

Table 2

Number of CFU-G-CSF		
Sample	Supernatant of BHK cells	Supernatant of BHK cells transfected with pDX-hJg1.Ex
Sample 1	99	34
Sample 2	48	20
Sample 3	45	23
Sample 4	48	19
Average	60	24

A cDNA fragment corresponding to the DSL region of hJAGGED1 (amino acids 178 to 240; SEQ ID NO:8) was amplified using primer 517 (SEQ ID NO:22; CGCGGATCCTCAGCCTTGTCGGCAAATAGC) and 518 (SEQ ID NO:23; CCCAAGCTTGCCCACTTTGAGTATCAGA). The fragment was subcloned into the PinPoint™ expression vector (Promega, Madison, WI), and expressed as a fusion protein with a peptide that becomes biotinylated in *E. coli*. After purification of the hJAGGED1 DSL fragment using avidin chromatography, the biotin-tagged hJAGGED1 fragment was assayed for activity in a high proliferative potential (HPP) assay with sorted mouse hematopoietic stem cells

(Sca-1$^+$, lin$^-$) as described in Patel et al., J. Exp. Med. 185:1163-1172 (1997), which is incorporated herein by reference). The HPP assay is an assay to test the self-renewal capacity of hematopoietic progenitor cells. Sorted mouse hematopoietic progenitor cells (Sca$^+$, lin$^-$) were cultured with a combination of growth factors (IL-1, IL-3 and stem cell factor) with or without 50-100 nM biotin-tagged hJAGGED1 DSL fragment SEQ ID NO:8 on soft agar for 10 days. The results of this assay demonstrated that the hJAGGED1 fragment SEQ ID NO:8 increased HPP efficiency two-fold. Thus, the hJAGGED1 fragment SEQ ID NO:8, corresponding to residues 178 to 240 of hJAGGED1, is an active fragment of JAGGED that increases the self-renewal capacity of hematopoietic progenitor cells.

EXAMPLE III

MAPPING hJAGGED1 RELATIVE TO THE ALAGILLE SYNDROME CRITICAL REGION

This example describes the mapping of the human JAGGED1 gene to chromosome 20p12.

hJAGGED1 Maps to Chromosome 20p12

In order to obtain a probe for fluorescence *in situ* hybridization (FISH), a total genomic library from Research Genetics (Huntsville, AL) was screened with the hJAGGED1 cDNA fragment Sdi-06. Two genomic bacterial artificial chromosome (BAC) clones, 49-D9 and 125-B1, were isolated, and the presence of the hJAGGED1 gene demonstrated by Southern blot analysis.

Probes were ^{32}P-labeled with PrimIt-II following the manufacturer's procedure (Stratagene, La Jolla, CA). Fluorescence *in situ* hybridization was performed with

each BAC clone independently. Both 49-D9 and 125-B1 hybridized specifically to 20p12 in a metaphase spread. FISH signals were observed at 20p12 on both chromosomes in each of the 10 metaphase cells analyzed and were not consistently observed at any other location. These results indicate that the hJAGGED1 gene maps to chromosome 20p12.

Fluorescence *in situ* hybridization was performed essentially as described in Trask, "Fluorescence in situ hybridization" in Birren et al., (Eds.) Genome Analysis: A Laboratory Manual Cold Spring Harbor Laboratory Press (1997) and Krantz, Am. J. Med Genet. 70:80-86 (1997), each of which is incorporated herein by reference. Briefly, BAC DNA was biotinylated by nick translation and hybridized to metaphase preparations (2 ng probe/μl). Human Cotl DNA (GIBCO-BRL) was added to the hybridization solution at a final concentration of 100 ng/ml to prevent hybridization of labeled repetitive sequences to chromosome spreads. Metaphase preparations were obtained from phytohemagglutinin-stimulated peripheral blood lymphocyte cultures that were blocked in early S-phase with methotrexate and released to (pro)metaphase in the presence of bromodeoxyuridine. Hybridization sites were detected with avidin-FITC, and chromosomes were banded with DAPI at 2 μg/ml in an antifade solution. FITC and DAPI images were collected separately, but in registration, using Spectrum Analytics IPLab Spectrum 3.0 software, a Princeton CCD camera (KAF 1400 chip), a Ludl filter-wheel equipped with ChromaTechnology excitation filters, and a Zeiss AxioPhot microscope equipped with a 100x, 13 N.A. objective and a ChromaTechnology multi-band pass emission filter. The images were pseudocolored and merged after the DAPI-banding contrast was enhanced by applying a 5x5 linear HAT filter supplied with the IPLab package. More than 10 metaphases were analyzed from the

computer screen or by direct visualization through the microscope.

Mapping hJAGGED1 relative to the Alagille Syndrome critical region.

Studies of the minimal region of overlap of multiple patients with cytogenetic deletions have defined an Alagille Syndrome critical region at chromosome 20p12 between genetic markers D20S41 and D20S162 (Figure 5). A contig of YAC, P1 and BAC clones spanning the critical region was used to further define this region. The distal boundary of the region is defined by a P1 clone (20p1-158), containing the synaptosomal associated protein-25 (SNAP-25). This clone was present in two copies in the patient with the most centromeric deletion (Krantz et al., *supra*, 1997). The centromeric boundary of the region is defined by P-1243b12, which is outside of the deletion in the patient with the most distal deletion. The size of this critical region is estimated at 1.2 to 1.3 Mb. Two BAC clones 49D9 and 125B1, which contain part of the hJAGGED1 gene, map to the 20p12 region. Using multiple PCR primers 249/250 (SEQ ID NOS: 24 and 25) and 247/248 (SEQ ID NOS:26 and 27) from BAC clone 49D9, on a panel of YAC, P1 and BAC clones, hJAGGED1 was sublocalized between D20S894 and D20S507 within the Alagille Syndrome critical region (see Figure 5).

CEPH human YAC clones were identified through the Whitehead Institute for Biomedical Research/MIT Center for Genomic Research web site and published data (Pollet et al., Genomics 27:467-474 (1995), which is incorporated herein by reference) and provided by Dr. Marcia Budarf (CHOP). The human P1 Library (Shepherd et al., Proc. Natl. Acad. Sci. 91:2629-2633 (1994), which is incorporated herein by reference) was screened

essentially as described in Stokke et al., Genomics 26:134-7 (1995), which is incorporated herein by reference. The human BAC library Stokke et al., *supra*, 1995; Shizuya et al., Proc. Natl. Acad. Sci. 89:8794-8797 (1992), which is incorporated herein by reference) was screened according to the protocol supplied by Research Genetics. Selected clones were mapped by FISH and STS content analysis to confirm cytogenetic localization and to order the clones. When clones were not contiguous, clone ends were obtained by sequencing using T7 and SP6 promoters, and new PCR primers were designed based on the sequence for the next round of library screening. Sequencing was carried out in the Nucleic Acid Sequencing Cores at the University of Pennsylvania, Department of Genetics, and at The Children's Hospital of Philadelphia. Fluorescence in situ hybridization studies were carried out by standard techniques essentially as described in, Krantz et al., *supra*, 1997, which is incorporated herein by reference.

Microsatellite markers were amplified as follows. $(TTTG)_n$ was amplified with primer pair 249/250 (GGTCTTTTGCCACTGTTT; SEQ ID NO:24 and GAATAGGGAGGAGAAAAC; SEQ ID NO:25), and $(GTTT)_n$ was amplified with primer pair 247/248 (GTCTTTTGCCACTGTTTG; SEQ ID NO:26 and GAATAGGGAGGAGAAAAC; SEQ ID NO:27).

EXAMPLE IV

hJAGGED1 GENE STRUCTURE

This example describes the identification of the hJAGGED exon/intron boundaries.

Identification of hJAGGED1 exon/intron boundaries

DNA array technology was used to determine the exon/intron boundaries of the hJAGGED1 gene as described in Nguyen et al., Genomics 29:207-216 (1995), which is incorporated herein by reference. BAC clone 49D9 was fragmented by sonication, and fragments ranging in size from 1.5 to 2 kb were selected and ligated into an M13 bacteriophage vector. Individual single stranded M13 clones were picked into 384-well microfilter plates, and 1,536 clones were arrayed onto four sets of nylon membranes using a 384-pin Replicator. The arrays of the BAC 49D9 M13 fragments were hybridized with the full length hJAGGED1 cDNA. All positive M13 clones (approximately 100 clones) were picked and sequenced. The hJAGGED1 genomic and cDNA sequences were aligned, and 47 intron/exon boundaries were defined (Figure 6A and 6B). The sequences from the 5' end, upstream of base pair 803 of the hJAGGED1 cDNA sequence, were missing one or two exons, presumably because the 5' end of the gene is not contained in the BAC 49D9 clone (Figure 6A). The 5' identified exons are indicated exon (n+1), where n stands for the unknown number of missing exons (see Figure 6B). The intron/exon and exon/intron boundary sequences of hJAGGED1 exons 3 through 26 are shown in Figure 6B as SEQ ID NOS:28 through 74.

BAC DNA sequence analysis was performed using random shot-gun sequencing essentially as described

above. Approximately 100 single-strand DNA templates were cloned into pCR.2.1 vector using the TA cloning system from Invitrogen. DNA was prepared using 5'-3' DNA mini-preparation system (5'prime-3'prime, Inc., Boulder, CO) and sequenced. Fluorescently-labeled -21M13 primer was used for sequencing of single-stranded DNA, and fluorescently labeled -21M13 and M13 forward primers were used for sequencing of double-stranded cDNA following the manufacture's procedure (ABI).

EXAMPLE V

ALAGILLE SYNDROME ASSOCIATED hJAGGED1 MUTATIONS

This example describes the association of several independent hJAGGED1 coding sequence mutations with Alagille Syndrome in four Alagille families.

Heteroduplex Mobility Analysis (HMA) of Alagille Syndrome Families

The hJAGGED1 gene contains at least 26 exons, and its mRNA is 5.5 kb in length. Heteroduplex mobility analysis (HMA) was used to screen for Alagille Syndrome-associated mutations in six RT-PCR products spanning the hJAGGED1 mRNA. HMA analysis is an assay that can readily detect mutations in heterozygotes at a given locus and is therefore potentially useful in screening for mutations in dominant disorders (Delwart et al., Science 262:1257-1261 (1993), which is incorporated herein by reference). Initially, ten individuals from four Alagille Syndrome families, each with multiple affected members, were screened by HMA (Figure 7). None of these families demonstrated deletions of 20p12 by cytogenetic or molecular analyses. RT-PCR was performed with six primer pairs to generate small overlapping cDNA fragments, designated A, B, C, D, E and F, which span

most of the hJAGGED1 coding sequence (Figure 7A). After localizing the mutation within one of the six amplified fragments, the cDNA region was sequenced and the identity of the mutation confirmed at the genomic level as described further below.

Shown in Figure 9 are the normal CNRAICRQGCS (SEQ ID NO:103) and corresponding mutant CNSYLPTRLQS* (SEQ ID NO:104) amino acid sequences of Alagille Syndrome family 1; the normal WCGPRPCL (SEQ ID NO:105) and corresponding mutant WCGVALDL (SEQ ID NO:106) amino acid sequences of Alagille Syndrome family 2; the normal DSQCD (SEQ ID NO:107) and corresponding mutant DSVMR (SEQ ID NO:108) amino acid sequences of Alagille Syndrome family 3; and the normal FCKCPED (SEQ ID NO:109) and corresponding mutant FCKCPRT (SEQ ID NO:110) amino acid sequences of Alagille Syndrome family 4.

Analysis of Alagille Syndrome Family 1

HMA analysis of family 1 indicated a mobility shift in PCR product "B" in two affected individuals (Figure 7B). Sequence analysis of the hJAGGED1 cDNAs from affected family members demonstrated a deletion of nucleotides "AG" at positions 1104 and 1105. To confirm that the two nucleotide deletion in the "B" region causes the mobility shift detected by HMA, cloned RT-PCR products from affected and unaffected family members were analyzed. cDNA with the "AG" deletion in combination with clones from a non-deleted individual produced an expected mobility shift identical to that of cDNAs from the RT-PCR products (Figure 7F and 7B). As anticipated, HMA analysis of each individual clone did not lead to the mobility shift. Fifteen cDNA clones from the "B" region were sequenced from each individual analyzed. Normal sequences were detected in all individuals in this family, but affected individuals demonstrated both mutant

and normal alleles. The "AG" deletion lies in exon (n+2).

Single strand conformational polymorphism (SSCP) analysis of exon 4 (designated exon n+2) on the extended family revealed a mobility shift in the three affected individuals in this family (Figure 8A). Furthermore, this deletion was confirmed by sequence analysis of the genomic DNA of exon (n+2) (Figure 8A). The disease-associated and normal nucleotide sequences of Alagille Syndrome family 1 in the region of this deletion are shown in Figure 8A as SEQ ID NOS:75 and 76, respectively. The "AG" deletion leads to a reading frame shift at residue 230, positioned at the end of the DSL domain, and is predicted to result in premature termination at residue 240. Thus, the "AG" deletion in family 1 results in a truncated hJAGGED1 protein lacking the 979 C-terminal residues (see Figure 9).

The two affected brothers in this family have liver disease, heart disease including pulmonic and peripheral pulmonic stenosis, posterior embryotoxon and Alagille facies. Their less severely affected mother has a heart murmur, posterior embryotoxon and Alagille facies.

Analysis of Alagille Syndrome Family 2

HMA analysis was similarly performed on family 2. PCR products from two affected members of family 2 showed mobility shifts in the "D" region (Figure 7C). cDNA sequence analysis of amplified "D" region sequences from both affected individuals revealed two changes: a five nucleotide insertion (GTGGC) at position 3102 and an 86 nucleotide deletion from nucleotides 2785 to 2871. The insertion is a repeat of the GTGGC sequence at positions 3102-3107. The 86 nucleotide deletion was

seen in all three members of this family, one of whom is unaffected, and corresponds to a complete absence of exon 23 (exon n+21). This result indicates that this exon can be removed from the final transcript by alternative splicing and that the 86 nucleotide deletion does not correlate with disease phenotype. Analyses in the "D" region of 10 individuals from four families identified a common heteroduplex. This observation is consistent with the presence of transcripts both containing and deleting exon (n+18) in all individuals tested (Figure 7B, C and D).

The multiple bands seen by HMA in the "D" region corresponded to the three types of variation identified by sequencing: a 5 bp insertion, a 86 bp deletion, and both a 5 bp insertion and an 86 bp deletion. Three cloned cDNA fragments, generated by PCR using the "D" region primers from individuals in Alagille Syndrome family 2, were tested. Each clone contains one variant. A clone from AGS2-2 (AGS $2\text{-}2_1$) contained the 5 nucleotide insertion. A clone from AGS 2-3 contained the 86 nucleotide deletion, and a third clone from AGS2-2 ($AGS2\text{-}2_2$) contained the 5 nucleotide insertion in addition to the 86 nucleotide deletion. These clones were hybridized with the normal clone D-n1 and analyzed by HMA. As shown in Figure 7E, these three types of hybridizations correspond to the heteroduplexes seen. These results indicate that only the five bp insertion correlates with the Alagille Syndrome disease phenotype. This disease-associated 5 bp insertion was localized to exon (n+21).

SSCP analysis revealed a novel band in this exon, present in an affected father and daughter and absent in the unaffected mother and in 50 normal control individuals (Figure 7C). The disease-associated and normal nucleotide sequences of Alagille Syndrome family 2

in the region of the mutation are shown in Figure 8B as SEQ ID NOS:77 and 78, respectively. The insertion, which was confirmed by genomic sequence analysis of the mutant hJAGGED1 genes in both affected individuals, is predicted to result in a translational frameshift downstream of codon 898. Translation is predicted to terminate at codon 945, resulting in a truncated hJAGGED1 protein lacking the C-terminal 274 residues. The mutant protein is predicted to contain the DSL domain, the entire EGF repeat domain, and about a third of the cysteine-rich domain, with an additional segment of 47 residues altered by the translational frameshift. The remainder of the cysteine-rich domain, the transmembrane (TM) domain and the intracellular region have been deleted (see Figure 9).

The phenotypes of the two affected individuals in this family are different. The father has liver disease, cardiac disease, and renal failure, while his daughter is more mildly affected with characteristic facies and pulmonary artery stenosis but normal liver and kidney function to date.

Analysis of Alagille Syndrome Family 3

The two affected individuals in this family showed shifts in the "C" region PCR products (Figure 7D). Sequence analysis revealed a four nucleotide "CAGT" deletion at positions 2531-2534 in exon (n+15) in both affected individuals. HMA analysis of a cDNA clone carrying the "CAGT" deletion, and a clone from a normal family member demonstrated a mobility shift (Figure 7F) identical to the RT-PCR products (Figure 7D).

SSCP analysis of exon (n+15) revealed a novel band in the affected proband, her affected mother, and in the DNA from the conceptus of a terminated pregnancy

(Figure 8C). The SSCP variant was not identified in 50 control individuals (100 chromosomes). The four nucleotide deletion was confirmed by genomic sequencing of exon 17 (exon n+15) from the affected individuals (Figure 8C). The disease-associated and normal nucleotide sequences of Alagille Syndrome family 3 in the region of the deletion are shown in Figure 8C as SEQ ID NOS:79 and 80, respectively. The mutant gene is predicted to encode an hJAGGED1 protein having a translational frameshift at residue 741 with an altered segment of 33 amino acids before chain termination. The translational frameshift occurs in the 12th EGF repeat as shown in Figure 9.

The proband in this family was severely affected, with liver involvement, severe branch pulmonary artery stenosis, butterfly vertebrae, and posterior embyrotoxon. She died at 2.5 years of age from head trauma after a fall. Her mother has a milder phenotype coming to medical attention at 20 years of age during pre-surgical evaluation for a basilar artery aneurysm. Studies at that time revealed abnormal liver function; further tests revealed bile duct paucity, pulmonic stenosis, characteristic facies and posterior embryotoxon with retinal changes.

Analysis of Alagille Syndrome Family 4

No heteroduplexes were seen in any of the six PCR products from individuals in this family (Figure 7D and 7F). However, cDNA sequence analysis revealed a single "C" nucleotide deletion at position 2066 in an affected daughter and father (Figure 7D). This deletion lies in exon (n+11).

SSCP analysis of exon (n+11) revealed an altered band in the proband and her father (Figure 8D).

Genomic sequence analyses verified the presence of the "C" deletion in exon 13 (exon n+11) in both affected family members. The disease-associated and normal nucleotide sequences of Alagille Syndrome family 4 in the region of the nucleotide deletion are shown in Figure 8D as SEQ ID NOS:81 and 82, respectively. The deletion shown in Figure 8D is predicted to result in a translational frameshift at residue 550 followed by an altered 13 residue segment before chain termination in EGF repeat 9 (Figure 9).

The proband was severely affected with liver and heart disease (tetralogy of Fallot), facial features of Alagille Syndrome, butterfly vertebrae and posterior embryotoxon. She died at 5 years of age from sepsis. Her father was mildly affected with a history of a heart murmur and characteristic facies. Liver studies were normal; an ophthalmology exam has yet to be conducted. The proband's sibling is also apparently affected, having severe congenital heart disease (tetralogy of Fallot) and posterior embyrotoxon. Her liver studies have been normal.

The Alagille Syndrome patients studied were subject to a complete diagnostic examination. All probands met the diagnostic criteria for the disorder. The proband of each family had Alagille syndrome as judged by the presence of bile duct paucity in addition to a minimum of three of the five following clinical criteria: cholestasis, cardiac disease, vertebral anomalies, anterior chamber defects of the eye and characteristic facial features. Additional family members were examined or their medical records reviewed. All patients and their families were enrolled in the study under an IRB approved protocol at the Children's Hospital of Philadelphia.

RT-PCR and Heteroduplex Mobility Analysis was performed as follows. Total RNA was isolated using Trizol RNA isolation kit (GIBCO-BRL), and cDNA was synthesized using GIBCO/BRL's reverse transcription system following the manufacture's procedure. Taq polymerase (Perkin Elmer) was used to amplify one-twentieth the volume of the reverse transcribed cDNA. The hJAGGED1 cDNA "A" segment was amplified with primers 292/395 (AGATCCTGTCCATGCAGAACGT; SEQ ID NO:83 and CATCCAGCCTTCCATGCAA; SEQ ID NO:84); the "B" segment was amplified with primers 398/399 (CTTTGAGTATCAGATCCGCGTGA; SEQ ID NO:85 and CGATGTCCAGCTGACAGA; SEQ ID NO:86); the "C" segment was amplified with primers 402/403 (CGGGATTTGGTTAATGGTTAT; SEQ ID NO:87 and GGTACCAGTTGTCTCCAT; SEQ ID NO:88); the "D" segment was amplified with primers 406/407 (GGAACAACCTGTAACATAGC; SEQ ID NO:89 and GGCCACATGTATTTCATTGTT; SEQ ID NO:90; the "E" segment was amplified with primers 408/409 (GAATATTCAATCTACATCGCTT; SEQ ID NO:91 and CTCAGACTCGAGTATGACACGA; SEQ ID NO:92); and the "F" segment was amplified with primers 410/411 (AAAGTGCCCAGAGCTTAAACCG; SEQ ID NO:93 and GGTGTTTTAAACATCTGACGTCGTA; SEQ ID NO:94).

Heteroduplex mobility analysis was performed using the following procedure: 200-500 ng of DNA was denatured at 96°C for five minutes in denaturing buffer (0.1M NaCl, 10 mM Tris HCl (pH 7.8), and 2 mM EDTA). The denatured DNA was immediately removed to a wet ice bath for five minutes and subsequently incubated at 55°C for five minutes. The reannealed DNA was mixed with loading buffer (0.2% Orange G, 2.5% Ficoll) and electrophoresed on a 5% polyacrylamide gel (19.5 X 19 cm) in 1X TBE buffer for 3 to 3.5 hours at 250 volts. After

electrophoresis, the gel was stained in 0.5 μg/ml ethidium bromide.

SSCP analysis was performed as follows. DNA was extracted from lymphocytes (whole blood) or established lymphoblastoid cell lines of affected and unaffected members of each Alagille family and from unrelated normal control subjects using the Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN). The primers for PCR analysis were designed to cover all exons as well as the intron/exon boundaries of hJAGGED1 as outlined in Figure 6B. For SSCP analysis, each PCR reaction contained 75 ng of genomic DNA, 200 μM dATP, dTTP, and dGTP, and 62.5 μM dCTP, 4 μCi of ^{32}P-dCTP, 10 pM of each primer, 1.0-1.5 mM $MgCl_2$, 2.5 μl dimethyl sulfoxide, 2.5 μl of 10X PCR Buffer II (Perkin Elmer, Foster City, CA), and 0.75 U AmpliTaq polymerase (Perkin Elmer) in a final volume of 25 μl. Exon (n+4) was amplified with primer pair 510/511 (CAGGGAAGAAGGCTGCAATGT; SEQ ID NO:95 and TGGTGGGGTGATAAATGGACAC; SEQ ID NO:96); exon (n+11) was amplified with primer pair 447/448 (GTTTTACTCTGATCCCTC; SEQ ID NO:97 and CAAGGGGCAGTGGTAGTAAGT; SEQ ID NO:98); exon (n+15) was amplified with primer pair 455/456 (GCTATCTCTGGGACCCTT; SEQ ID NO:99 and CCACGTGGGGCATAAAGTT; SEQ ID NO:100); and exon (n+21) was amplified with primer pair 467/468 (ATGGCTGCCGCAGTTCA; SEQ ID NO:101 and CAAGCAGACATCCACCAT; SEQ ID NO:102). PCR conditions were as follows: 94°C, 30 seconds; 50°C, 1 minute; and 72°C, 30 seconds for 35 cycles.

The denatured PCR products were analyzed by electrophoresis on MDE gels (FMC Corp., Pinebrook, NJ) with and without glycerol at 4°C for 4-5 hours. Gels were transferred to filter paper and exposed to X-ray film at 70°C for 1 to 24 hours. Amplicons demonstrating SSCP band shifts were sequenced by the Nucleic

'Acid/Protein core facility of the Children's Hospital of 'Philadelphia using an ABI373A automated sequencer.

All journal article, reference, and patent citations provided above, in parentheses or otherwise, whether previously stated or not, are incorporated herein by reference.

Although the invention has been described with reference to the disclosed embodiments, those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention. It should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:

1. An isolated JAGGED peptide having at most about 40 amino acids, comprising substantially the same amino acid sequence as SEQ ID NO:9.

2. The isolated JAGGED peptide of claim 1, comprising the amino acid sequence SEQ ID NO:9.

3. The isolated JAGGED peptide of claim 2, consisting of the amino acid sequence SEQ ID NO:9.

4. A method of inhibiting differentiation of hematopoietic progenitor cells, comprising contacting said progenitor cells with an isolated JAGGED polypeptide comprising substantially the same amino acid sequence as JAGGED, or an active fragment thereof.

5. The method of claim 4, wherein said progenitor cells are contacted *in vitro*.

6. The method of claim 4, wherein said isolated JAGGED polypeptide comprises substantially the same amino acid sequence as SEQ ID NO:2 or SEQ ID NO:4, or an active fragment thereof.

7. The method of claim 6, wherein said active fragment is a soluble fragment.

8. The method of claim 7, wherein said soluble fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NO:7 and SEQ ID NO:8.

9. A method of inhibiting differentiation of progenitor cells, comprising contacting said progenitor cells with an isolated JAGGED peptide having at most about 40 amino acids and comprising substantially the same amino acid sequence as SEQ ID NO:9.

10. The method of claim 9, wherein said cells are hematopoietic progenitor cells.

11. The method of claim 9, wherein said cells are contacted *in vitro*.

12. The method of claim 9, wherein said isolated JAGGED peptide comprises the amino acid sequence SEQ ID NO:9.

13. The method of claim 12, wherein said isolated JAGGED peptide consists of the amino acid sequence SEQ ID NO:9.

14. A method of maintaining progenitor cells in an undifferentiated state, comprising contacting said progenitor cells with a JAGGED polypeptide, or active fragment thereof.

15. The method of claim 14, wherein said progenitor cells are capable of reconstituting the hematopoietic system.

16. The method of claim 14, wherein said progenitor cells are maintained in a totipotent state.

17. The method of claim 16, wherein said progenitor cells are maintained in a totipotent state in culture.

18. The method of claim 14, further comprising cryopreservation of said progenitor cells maintained in an undifferentiated state.

19. The method of claim 14, further comprising introducing a nucleic acid molecule encoding a gene product into said progenitor cells.

20. A method of maintaining progenitor cells in an undifferentiated state, comprising contacting said progenitor cells with a JAGGED peptide having at most about 40 amino acids and comprising substantially the same amino acid sequence as SEQ ID NO:9.

21. The method of claim 20, wherein said progenitor cells are capable of reconstituting the hematopoietic system.

22. The method of claim 20, wherein said progenitor cells are maintained in a totipotent state.

23. The method of claim 22, wherein said progenitor cells are maintained in a totipotent state in culture.

24. The method of claim 20, further comprising cryopreservation of said progenitor cells maintained in an undifferentiated state.

25. The method of claim 20, further comprising introducing a nucleic acid molecule encoding a gene product into said progenitor cells.

26. A method of diagnosing Alagille Syndrome in an individual, comprising detecting an Alagille Syndrome disease-associated mutation linked to a JAGGED locus.

27. The method of claim 26, wherein said disease-associated mutation is within a JAGGED gene.

28. The method of claim 27, wherein said disease-associated mutation is within a JAGGED coding sequence.

29. The method of claim 26, wherein said JAGGED locus is a human JAGGED1 (hJAGGED1) locus.

30. The method of claim 29, wherein said disease-associated mutation produces a truncated hJAGGED1 gene product.

31. The method of claim 30, wherein said disease-associated mutation occurs within the hJAGGED1 nucleotide sequence SEQ ID NO:1 at a position selected from group consisting of nucleotides 1104-1105, nucleotide 3102, nucleotides 2531-2534 and nucleotide 2066.

```
                CTGCGGCCGGCCCGCGAGCTAGGCTGGGTTTTTTTTTTCTCCCCTCCCTCCCCCCTTTT
1               ---------+---------+---------+---------+---------+---------+  60

                TCCATGCAGCTGATCTAAAAGGGAATAAAAGGCTGCGCATAATCATAATAATAAAAGAAG
61              ---------+---------+---------+---------+---------+---------+ 120

                GGGAGCGCGAGAGAAGGAAAGAAAGCCGGGAGGTGGAAGAGGAGGGGGAGCGTCTCAAAG
121             ---------+---------+---------+---------+---------+---------+ 180

                AAGCGATCAGAATAATAAAAGGAGGCCGGGCTCTTTGCCTTCTGGAACGGGCCGCTCTTG
181             ---------+---------+---------+---------+---------+---------+ 240

                AAAGGGCTTTTGAAAAGTGGTGTTGTTTTCCAGTCGTGCATGCTCCAATCGGCGGAGTAT
241             ---------+---------+---------+---------+---------+---------+ 300

                ATTAGAGCCGGGACGCGGCGGCCGCAGGGGCAGCGGCGACGGCAGCACCGGCGGCAGCAC
301             ---------+---------+---------+---------+---------+---------+ 360

                CAGCGCGAACAGCAGCGGCGGCGTCCCGAGTGCCCGCGGCGCGCGGCGCAGCGATGCGTT
361             ---------+---------+---------+---------+---------+---------+ 420

                                                                  M  R  S

                CCCCACGGACGCGCGGCCGGTCCGGGCGCCCCCTAAGCCTCCTGCTCGCCCTGCTCTGTG
421             ---------+---------+---------+---------+---------+---------+ 480

                  P  R  T  R  G  R  S  G  R  P  L  S  L  L  L  A  L  L  C  A

                CCCTGCGAGCCAAGGTGTGTGGGGCCTCGGGTCAGTTCGAGTTGGAGATCCTGTCCATGC
481             ---------+---------+---------+---------+---------+---------+ 540

                  L  R  A  K  V  C  G  A  S  G  Q  F  E  L  E  I  L  S  M  Q

                AGAACGTGAACGGGGAGCTGCAGAACGGGAACTGCTGCGGCGGCGCCCGGAACCCGGGAG
541             ---------+---------+---------+---------+---------+---------+ 600

                  N  V  N  G  E  L  Q  N  G  N  C  C  G  G  A  R  N  P  G  D

                ACCGCAAGTGCACCCGCGACGAGTGTGACACATACTTCAAAGTGTGCCTCAAGGAGTATC
601             ---------+---------+---------+---------+---------+---------+ 660

                  R  K  C  T  R  D  E  C  D  T  Y  F  K  V  C  L  K  E  Y  Q

                AGTCCCGCGTCACGGCCGGGGGGCCCTGCAGCTTCGGCTCAGGGTCCACGCCTGTCATCG
661             ---------+---------+---------+---------+---------+---------+ 720

                  S  R  V  T  A  G  G  P  C  S  F  G  S  G  S  T  P  V  I  G
```

FIG. 1A-1

```
      GGGGCAACACCTTCAACCTCAAGGCCAGCCGCGGCAACGACCGCAACCGCATCGTGCTGC
721   ---------+---------+---------+---------+---------+---------+  780
        G  N  T  F  N  L  K  A  S  R  G  N  D  R  N  R  I  V  L  P

      CTTTCAGTTTCGCCTGGCCGAGGTCCTATACGTTGCTTGTGGAGGCGTGGGATTCCAGTA
781   ---------+---------+---------+---------+---------+---------+  840
        F  S  F  A  W  P  R  S  Y  T  L  L  V  E  A  W  D  S  S  N

      ATGACACCGTTCAACCTGACAGTATTATTGAAAAGGCTTCTCACTCGGGCATGATCAACC
841   ---------+---------+---------+---------+---------+---------+  900
        D  T  V  Q  P  D  S  I  I  E  K  A  S  H  S  G  M  I  N  P

      CCAGCCGGCAGTGGCAGACGCTGAAGCAGAACACGGGCGTTGCCCACTTTGAGTATCAGA
901   ---------+---------+---------+---------+---------+---------+  960
        S  R  Q  W  Q  T  L  K  Q  N  T  G  V  A  H  F  E  Y  Q  I

      TCCGCGTGACCTGTGATGACTACTACTATGGCTTTGGCTGCAATAAGTTCTGCCGCCCCA
961   ---------+---------+---------+---------+---------+---------+ 1020
        R  V  T  C  D  D  Y  Y  Y  G  F  G  C  N  K  F  C  R  P  R

      GAGATGACTTCTTTGGACACTATGCCTGTGACCAGAATGGCAACAAAACTTGCATGGAAG
1021  ---------+---------+---------+---------+---------+---------+ 1080
        D  D  F  F  G  H  Y  A  C  D  Q  N  G  N  K  T  C  M  E  G

      GCTGGATGGGCCCCGAATGTAACAGAGCTATTTGCCGACAAGGCTGCAGTCCTAAGCATG
1081  ---------+---------+---------+---------+---------+---------+ 1140
        W  M  G  P  E  C  N  R  A  I  C  R  Q  G  C  S  P  K  H  G

      GGTCTTGCAAACTCCCAGGTGACTGCAGGTGCCAGTATGGCTGGCAAGGCCTGTACTGTG
1141  ---------+---------+---------+---------+---------+---------+ 1200
        S  C  K  L  P  G  D  C  R  C  Q  Y  G  W  Q  G  L  Y  C  D

      ATAAGTGCATCCCACACCCGGGATGCGTCCACGGCATCTGTAATGAGCCCTGGCAGTGCC
1201  ---------+---------+---------+---------+---------+---------+ 1260
        K  C  I  P  H  P  G  C  V  H  G  I  C  N  E  P  W  Q  C  L
```

FIG. 1A-2

```
                TCTGTGAGACCAACTGGGGCGGCCAGCTCTGTGACAAAGATCTCAATTACTGTGGGACTC
1261  --------+--------+--------+--------+--------+--------+--------+  1320

                  C  E  T  N  W  G  G  Q  L  C  D  K  D  L  N  Y  C  G  T  H

                ATCAGCCGTGTCTCAACGGGGGAACTTGTAGCAACACAGGCCCTGACAAATATCAGTGTT
1321  --------+--------+--------+--------+--------+--------+--------+  1380

                  Q  P  C  L  N  G  G  T  C  S  N  T  G  P  D  K  Y  Q  C  S

                CCTGCCCTGAGGGGTATTCAGGACCCAACTGTGAAATTGCTGAGCACGCCTGCCTCTCTG
1381  --------+--------+--------+--------+--------+--------+--------+  1440

                  C  P  E  G  Y  S  G  P  N  C  E  I  A  E  H  A  C  L  S  D

                ATCCCTGTCACAACAGAGGCAGCTGTAAGGAGACCTCCCTGGGCTTTGAGTGTGAGTGTT
1441  --------+--------+--------+--------+--------+--------+--------+  1500

                  P  C  H  N  R  G  S  C  K  E  T  S  L  G  F  E  C  E  C  S

                CCCCAGGCTGGACCGGCCCCACATGCTCTACAAACATTGATGACTGTTCTCCTAATAACT
1501  --------+--------+--------+--------+--------+--------+--------+  1560

                  P  G  W  T  G  P  T  C  S  T  N  I  D  D  C  S  P  N  N  C

                GTTCCCACGGGGGCACCTGCCAGGACCTGGTTAACGGATTTAAGTGTGTGTGCCCCCCAC
1561  --------+--------+--------+--------+--------+--------+--------+  1620

                  S  H  G  G  T  C  Q  D  L  V  N  G  F  K  C  V  C  P  P  Q

                AGTGGACTGGGAAAACGTGCCAGTTAGATGCAAATGAATGTGAGGCCAAACCTTGTGTAA
1621  --------+--------+--------+--------+--------+--------+--------+  1680

                  W  T  G  K  T  C  Q  L  D  A  N  E  C  E  A  K  P  C  V  N

                ACGCCAAATCCTGTAAGAATCTCATTGCCAGCTACTACTGCGACTGTCTTCCCGGCTGGA
1681  --------+--------+--------+--------+--------+--------+--------+  1740

                  A  K  S  C  K  N  L  I  A  S  Y  Y  C  D  C  L  P  G  W  M

                TGGGTCAGAATTGTGACATAAATATTAATGACTGCCTTGGCCAGTGTCAGAATGACGCCT
1741  --------+--------+--------+--------+--------+--------+--------+  1800

                  G  Q  N  C  D  I  N  I  N  D  C  L  G  Q  C  Q  N  D  A  S

                CCTGTCGGGATTTGGTTAATGGTTATCGCTGTATCTGTCCACCTGGCTATGCAGGCGATC
1801  --------+--------+--------+--------+--------+--------+--------+  1860

                  C  R  D  L  V  N  G  Y  R  C  I  C  P  P  G  Y  A  G  D  H
```

FIG. 1A-3

```
                ACTGTGAGAGAGACATCGATGAATGTGCCAGCAACCCCTGTTTGAATGGGGGTCACTGTC
1861  ---------+---------+---------+---------+---------+---------+---------+  1920

                  C  E  R  D  I  D  E  C  A  S  N  P  C  L  N  G  G  H  C  Q

                AGAATGAAATCAACAGATTCCAGTGTCTGTGTCCCACTGGTTTCTCTGGAAACCTCTGTC
1921  ---------+---------+---------+---------+---------+---------+---------+  1980

                  N  E  I  N  R  F  Q  C  L  C  P  T  G  F  S  G  N  L  C  Q

                AGCTGGACATCGATTATTGTGAGCCTAATCCCTGCCAGAACGGTGCCCAGTGCTACAACC
1981  ---------+---------+---------+---------+---------+---------+---------+  2040

                  L  D  I  D  Y  C  E  P  N  P  C  Q  N  G  A  Q  C  Y  N  R

                TGAAAGACCACTGCCGCACGACCCCCTGTGAAGTGATTGACAGCTGCACAGTGGCCATGG
2041  ---------+---------+---------+---------+---------+---------+---------+  2100

                  A  S  D  Y  F  C  K  C  P  E  D  Y  E  G  K  N  C  S  H  L

                TGAAAGACCACTGCCGCACGACCCCCTGTGAAGTGATTGACAGCTGCACAGTGGCCATGG
2101  ---------+---------+---------+---------+---------+---------+---------+  2160

                  K  D  H  C  R  T  T  P  C  E  V  I  D  S  C  T  V  A  M  A

                CTTCCAACGACACACCTGAAGGGGTGCGGTATATTTCCTCCAACGTCTGTGGTCCTCACG
2161  ---------+---------+---------+---------+---------+---------+---------+  2220

                  S  N  D  T  P  E  G  V  R  Y  I  S  S  N  V  C  G  P  H  G

                GGAAGTGCAAGAGTCAGTCGGGAGGCAAATTCACCTGTGACTGTAACAAAGGCTTCACGG
2221  ---------+---------+---------+---------+---------+---------+---------+  2280

                  K  C  K  S  Q  S  G  G  K  F  T  C  D  C  N  K  G  F  T  G

                GAACATACTGCCATGAAAATATTAATGACTGTGAGAGCAACCCTTGTAGAAACGGTGGCA
2281  ---------+---------+---------+---------+---------+---------+---------+  2340

                  T  Y  C  H  E  N  I  N  D  C  E  S  N  P  C  R  N  G  G  T

                CTTGCATCGATGGTGTCAACTCCTACAAGTGCATCTGTAGTGACGGCTGGGAGGGGGCCT
2341  ---------+---------+---------+---------+---------+---------+---------+  2400

                  C  I  D  G  V  N  S  Y  K  C  I  C  S  D  G  W  E  G  A  Y
```

FIG. 1A-4

```
                ACTGTGAAACCAATATTAATGACTGCAGCCAGAACCCCTGCCACAATGGGGGCACGTGTC
2401            ---------+---------+---------+---------+---------+---------+  2460

                  C   E   T   N   I   N   D   C   S   Q   N   P   C   H   N   G   G   T   C   R

                GCGACCTGGTCAATGACTTCTACTGTGACTGTAAAAATGGGTGGAAAGGAAAGACCTGCC
2461            ---------+---------+---------+---------+---------+---------+  2520

                  D   L   V   N   D   F   Y   C   D   C   K   N   G   W   K   G   K   T   C   H

                ACTCACGTGACAGTCAGTGTGATGAGGCCACGTGCAACAACGGTGGCACCTGCTATGATG
2521            ---------+---------+---------+---------+---------+---------+  2580

                  S   R   D   S   Q   C   D   E   A   T   C   N   N   G   G   T   C   Y   D   E

                AGGGGGATGCTTTTAAGTGCATGTGTCCTGGCGGCTGGGAAGGAACAACCTGTAACATAG
2581            ---------+---------+---------+---------+---------+---------+  2640

                  G   D   A   F   K   C   M   C   P   G   G   W   E   G   T   T   C   N   I   A

                CCCGAAACAGTAGCTGCCTGCCCAACCCCTGCCATAATGGGGGCACATGTGTGGTCAACG
2641            ---------+---------+---------+---------+---------+---------+  2700

                  R   N   S   S   C   L   P   N   P   C   H   N   G   G   T   C   V   V   N   G

                GCGAGTCCTTTACGTGCGTCTGCAAGGAAGGCTGGGAGGGGCCCATCTGTGCTCAGAATA
2701            ---------+---------+---------+---------+---------+---------+  2760

                  E   S   F   T   C   V   C   K   E   G   W   E   G   P   I   C   A   Q   N   T

                CCAATGACTGCAGCCCTCATCCCTGTTACAACAGCGGCACCTGTGTGGATGGAGACAACT
2761            ---------+---------+---------+---------+---------+---------+  2820

                  N   D   C   S   P   H   P   C   Y   N   S   G   T   C   V   D   G   D   N   W

                GGTACCGGTGCGAATGTGCCCCGGGTTTTGCTGGGCCCGACTGCAGAATAAACATCAATG
2821            ---------+---------+---------+---------+---------+---------+  2880

                  Y   R   C   E   C   A   P   G   F   A   G   P   D   C   R   I   N   I   N   E

                AATGCCAGTCTTCACCTTGTGCCTTTGGAGCGACCTGTGTGGATGAGATCAATGGCTACC
2881            ---------+---------+---------+---------+---------+---------+  2940

                  C   Q   S   S   P   C   A   F   G   A   T   C   V   D   E   I   N   G   Y   R

                GGTGTGTCTGCCCTCCAGGGCACAGTGGTGCCAAGTGCCAGGAAGTTTCAGGGAGACCTT
2941            ---------+---------+---------+---------+---------+---------+  3000

                  C   V   C   P   P   G   H   S   G   A   K   C   Q   E   V   S   G   R   P   C
```

FIG. 1A-5

```
                GCATCACCATGGGGAGTGTGATACCAGATGGGGCCAAATGGGATGATGACTGTAATACCT
3001  ---------+---------+---------+---------+---------+---------+---------+  3060
                 I  T  M  G  S  V  I  P  D  G  A  K  W  D  D  D  C  N  T  C

                GCCAGTGCCTGAATGGACGGATCGCCTGCTCAAAGGTCTGGTGTGGCCCTCGACCTTGCC
3061  ---------+---------+---------+---------+---------+---------+---------+  3120
                 Q  C  L  N  G  R  I  A  C  S  K  V  W  C  G  P  R  P  C  L

                TGCTCCACAAAGGGCACAGCGAGTGCCCCAGCGGGCAGAGCTGCATCCCCATCCTGGACG
3121  ---------+---------+---------+---------+---------+---------+---------+  3180
                 L  H  K  G  H  S  E  C  P  S  G  Q  S  C  I  P  I  L  D  D

                ACCAGTGCTTCGTCCACCCCTGCACTGGTGTGGGCGAGTGTCGGTCTTCCAGTCTCCAGC
3181  ---------+---------+---------+---------+---------+---------+---------+  3240
                 Q  C  F  V  H  P  C  T  G  V  G  E  C  R  S  S  S  L  Q  P

                CGGTGAAGACAAAGTGCACCTCTGACTCCTATTACCAGGATAACTGTGCGAACATCACAT
3241  ---------+---------+---------+---------+---------+---------+---------+  3300
                 V  K  T  K  C  T  S  D  S  Y  Y  Q  D  N  C  A  N  I  T  F

                TTACCTTTAACAAGGAGATGATGTCACCAGGTCTTACTACGGAGCACATTTGCAGTGAAT
3301  ---------+---------+---------+---------+---------+---------+---------+  3360
                 T  F  N  K  E  M  M  S  P  G  L  T  T  E  H  I  C  S  E  L

                TGAGGAATTTGAATATTTTGAAGAATGTTTCCGCTGAATATTCAATCTACATCGCTTGCG
3361  ---------+---------+---------+---------+---------+---------+---------+  3420
                 R  N  L  N  I  L  K  N  V  S  A  E  Y  S  I  Y  I  A  C  E

                AGCCTTCCCCTTCAGCGAACAATGAAATACATGTGGCCATTTCTGCTGAAGATATACGGG
3421  ---------+---------+---------+---------+---------+---------+---------+  3480
                 P  S  P  S  A  N  N  E  I  H  V  A  I  S  A  E  D  I  R  D

                ATGATGGGAACCCGATCAAGGAAATCACTGACAAAATAATCGATCTTGTTAGTAAACGTG
3481  ---------+---------+---------+---------+---------+---------+---------+  3540
                 D  G  N  P  I  K  E  I  T  D  K  I  I  D  L  V  S  K  R  D

                ATGGAAACAGCTCGCTGATTGCTGCCGTTGCAGAAGTAAGAGTTCAGAGGCGGCCTCTGA
3541  ---------+---------+---------+---------+---------+---------+---------+  3600
                 G  N  S  S  L  I  A  A  V  A  E  V  R  V  Q  R  R  P  L  K
```

FIG. 1A-6

```
                AGAACAGAACAGATTTCCTTGTTCCCTTGCTGAGCTCTGTCTTAACTGTGGCTTGGATCT
3601  ---------+---------+---------+---------+---------+---------+---------+  3660

                  N  R  T  D  F  L  V  P  L  L  S  S  V  L  T  V  A  W  I  C

                GTTGCTTGGTGACGGCCTTCTACTGGTGCCTGCGGAAGCGGCGGAAGCCGGGCAGCCACA
3661  ---------+---------+---------+---------+---------+---------+---------+  3720

                  C  L  V  T  A  F  Y  W  C  L  R  K  R  R  K  P  G  S  H  T

                CACACTCAGCCTCTGAGGACAACACCACCAACAACGTGCGGGAGCAGCTGAACCAGATCA
3721  ---------+---------+---------+---------+---------+---------+---------+  3780

                  H  S  A  S  E  D  N  T  T  N  N  V  R  E  Q  L  N  Q  I  K

                AAAACCCCATTGAGAAACATGGGGCCAACACGGTCCCCATCAAGGATTACGAGAACAAGA
3781  ---------+---------+---------+---------+---------+---------+---------+  3840

                  N  P  I  E  K  H  G  A  N  T  V  P  I  K  D  Y  E  N  K  N

                ACTCCAAAATGTCTAAAATAAGGACACACAATTCTGAAGTAGAAGAGGACGACATGGACA
3841  ---------+---------+---------+---------+---------+---------+---------+  3900

                  S  K  M  S  K  I  R  T  H  N  S  E  V  E  E  D  D  M  D  K

                AACACCAGCAGAAAGCCCGGTTTGCCAAGCAGCCGGCGTATACGCTGGTAGACAGAGAAG
3901  ---------+---------+---------+---------+---------+---------+---------+  3960

                  H  Q  Q  K  A  R  F  A  K  Q  P  A  Y  T  L  V  D  R  E  E

                AGAAGCCCCCCAACGGCACGCCGACAAAACACCCAAACTGGACAAACAAACAGGACAACA
3961  ---------+---------+---------+---------+---------+---------+---------+  4020

                  K  P  P  N  G  T  P  T  K  H  P  N  W  T  N  K  Q  D  N  R

                GAGACTTGGAAAGTGCCCAGAGCTTAAACCGAATGGAGTACATCGTATAGCAGACCGCGG
4021  ---------+---------+---------+---------+---------+---------+---------+  4080

                  D  L  E  S  A  Q  S  L  N  R  M  E  Y  I  V  *

                GCACTGCCGCCGCTAGGTAGAGTCTGAGGGCTTGTAGTTCTTTAAACTGTCGTGTCATAC
4081  ---------+---------+---------+---------+---------+---------+---------+  4140


                TCGAGTCTGAGGCCGTTGCTGACTTAGAATCCCTGTGTTAATTTAAGTTTTGACAAGCTG
4141  ---------+---------+---------+---------+---------+---------+---------+  4200


                GCTTACACTGGCAATGGTAGTTTCTGTGGTTGGCTGGGAAATCGAGTGCCGCATCTCACA
4201  ---------+---------+---------+---------+---------+---------+---------+  4260
```

FIG. 1A-7

```
GCTATGCAAAAAGCTAGTCAACAGTACCCTGGTTGTGTGTCCCCTTGCAGCCGACACGGT
4261 ---------+---------+---------+---------+---------+---------+ 4320

     CTCGGATCAGGCTCCCAGGAGCCTGCCCAGCCCCCTGGTCTTTGAGCTCCCACTTCTGCC
4321 ---------+---------+---------+---------+---------+---------+ 4380

     AGATGTCCTAATGGTGATGCAGTCTTAGATCATAGTTTTATTTATATTTATTGACTCTTG
4381 ---------+---------+---------+---------+---------+---------+ 4440

     AGTTGTTTTTGTATATTGGTTTTATGATGACGTACAAGTAGTTCTGTATTTGAAAGTGCC
4441 ---------+---------+---------+---------+---------+---------+ 4500

     TTTGCAGCTCAGAACCACAGCAACGATCACAAATGACTTTATTATTTATTTTTTTAATTG
4501 ---------+---------+---------+---------+---------+---------+ 4560

     TATTTTTGTTGTTGGGGGAGGGGAGACTTTGATGTCAGCAGTTGCTGGTAAAATGAAGAA
4561 ---------+---------+---------+---------+---------+---------+ 4620

     TTTAAAGAAAAAAAATGTCAAAAGTAGAACTTTGTATAGTTATGTAAATAATTCTTTTTTA
4621 ---------+---------+---------+---------+---------+---------+ 4680

     TTAATCACTGTGTATATTTGATTTATTAACTTAATAATCAAGAGCCTTAAAACATCATTC
4681 ---------+---------+---------+---------+---------+---------+ 4740

     CTTTTTATTTATATGTATGTGTTTAGAATTGAAGGTTTTTGATAGCATTGTAAGCGTATG
4741 ---------+---------+---------+---------+---------+---------+ 4800

     GCTTTATTTTTTTGAACTCTTCTCATTACTTGTTGCCTATAAGCCAAAATTAAGGTGTTT
4801 ---------+---------+---------+---------+---------+---------+ 4860

     GAAAATAGTTTATTTTAAAACAATAGGATGGGCTTCTGTGCCCAGAATACTGATGGAATT
4861 ---------+---------+---------+---------+---------+---------+ 4920

     TTTTTTGTACGACGTCAGATGTTTAAAACACCTTCTATAGCATCACTTAAAACACGTTTT
4921 ---------+---------+---------+---------+---------+---------+ 4980

     AAGGACTGACTGAGGCAGTTTGAGGATTAGTTTAGAACAGGTTTTTTTGTTTGTTTGTTT
4981 ---------+---------+---------+---------+---------+---------+ 5040
```

FIG. 1A-8

```
                TTTGTTTTTCTGCTTTAGACTTGAAAAGAGACAGGCAGGTGATCTGCTGCAGAGCAGTAA
5041  ---------+---------+---------+---------+---------+---------+---------+  5100

                GGGAACAAGTTGAGCTATGACTTAACATAGCCAAAATGTGAGTGGTTGAATATGATTAAA
5101  ---------+---------+---------+---------+---------+---------+---------+  5160

                AATATCAAATTAATTGTGTGAACTTGGAAGCACACCAATCTGACTTTGTAAATTCTGATT
5161  ---------+---------+---------+---------+---------+---------+---------+  5220

                TCTTTTCACCATTCGTACATAATACTGAACCACTTGTAGATTTGATTTTTTTTTTAATCT
5221  ---------+---------+---------+---------+---------+---------+---------+  5280

                ACTGCATTTAGGGAGTATTCTAATAAGCTAGTTGAATACTTGAACCATAAAATGTCCAGT
5281  ---------+---------+---------+---------+---------+---------+---------+  5340

                AAGATCACTGTTTAGATTTGCCATAGAGTACACTGCCCTGCCTTAAGTGAGGAAATCAAG
5341  ---------+---------+---------+---------+---------+---------+---------+  5400

                TGCTATTACGAAGTTCAAGATCAAAAAGGCTTATAAAACAGAGTAATCTTGTTGGTTCAC
5401  ---------+---------+---------+---------+---------+---------+---------+  5460

                CATTGAGACCGTGAAGATACTTTGTATTGTCCTATTAGTGTTATATGAACATACAAATGC
5461  ---------+---------+---------+---------+---------+---------+---------+  5520

                ATCTTTGATGTGTTGTTCTTGGCAATAAATTTTGAAAAGTAATATTTATTAAATTTTTTT
5521  ---------+---------+---------+---------+---------+---------+---------+  5580

                GTATGAAAAC
5581  ---------+  5590
```

FIG. 1A-9

```
                CGGCCGCGTCGACGTGACGGCGACGGCCGGACAACGCGCGCGGGGGGCTGCGGCCACGAC
          1     ---------+---------+---------+---------+---------+---------+     60

                          D  G  D  G  R  T  T  R  A  G  G  C  G  H  D

                GAGTGCGACACGTACGTGCGCGTGTGCCTTAAGGAGTACCAGGCCAAGGTGACGCCCACG
         61     ---------+---------+---------+---------+---------+---------+    120

                E  C  D  T  Y  V  R  V  C  L  K  E  Y  Q  A  K  V  T  P  T

                GGGCCCTGCAGCTACGGCCACGGCGCCACGCCCGTGCTGGGCGGCAACTCCTTCTACCTG
        121     ---------+---------+---------+---------+---------+---------+    180

                G  P  C  S  Y  G  H  G  A  T  P  V  L  G  G  N  S  F  Y  L

                CCGCCGGCGGGCGCTGCGGGGGACCGAGCGCGGGCGCGGGCCCGGGCCGGCGGCGACCAG
        181     ---------+---------+---------+---------+---------+---------+    240

                P  P  A  G  A  A  G  D  R  A  R  A  R  A  R  A  G  G  D  Q

                GACCCGGGCCTCGTCGTCATCCCCTTCCAGTTCGCCTGGCCGCGCTCCTTTACCCTCATC
        241     ---------+---------+---------+---------+---------+---------+    300
                                                                      GGAGTAG
                D  P  G  L  V  V  I  P  F  Q  F  A  W  P  R  S  F  T  L  I

                GTGGAGGCCTGGGACTGGGACAACGATACCACCCCGAATGAGGAGCTGCTGATCGAGCGA
        301     ---------+---------+---------+---------+---------+---------+    360

                V  E  A  W  D  W  D  N  D  T  T  P  N  E  E  L  L  I  E  R

                GTGTCGCATGCCGGCATGATCAACCCGGAGGACCGCTGGAAGAGCCTGCACTTCAGCGGC
        361     ---------+---------+---------+---------+---------+---------+    420

                V  S  H  A  G  M  I  N  P  E  D  R  W  K  S  L  H  F  S  G

                CACGTGGCGCACCTGGAGCTGCAGATCCGCGTGCGCTGCGACGAGAACTACTACAGCGCC
        421     ---------+---------+---------+---------+---------+---------+    480

                H  V  A  H  L  E  L  Q  I  R  V  R  C  D  E  N  Y  Y  S  A

                ACTTGCAACAAGTTCTGCCGGCCCCGCAACGACTTTTTCGGCCACTACACCTGCGACCAG
        481     ---------+---------+---------+---------+---------+---------+    540

                T  C  N  K  F  C  R  P  R  N  D  F  F  G  H  Y  T  C  D  Q

                TACGGCAACAAGGCCTGCATGGACGGCTGGATGGGCAAGGAGTGCAAGGAAGCTGTGTGT
        541     ---------+---------+---------+---------+---------+---------+    600

                Y  G  N  K  A  C  M  D  G  W  M  G  K  E  C  K  E  A  V  C
```

FIG. 1B-1

```
      AAACAAGGGTGTAATTTGCTCCACGGGGGATGCACCGTGCCTGGGGAGTGCAGGTGCAGC
601   ---------+---------+---------+---------+---------+---------+   660

      K  Q  G  C  N  L  L  H  G  G  C  T  V  P  G  E  C  R  C  S

      TACGGCTGGCAAGGGAGGTTCTGCGATGAGTGTGTCCCCTACCCCGGCTGCGTGCATGGC
661   ---------+---------+---------+---------+---------+---------+   720
      Y  G  W  Q  G  R  F  C  D  E  C  V  P  Y  P  G  C  V  H  G

      AGTTGTGTGGAGCCCTGGCAGTGCAACTGTGAGACCAACTGGGGCGGCCTGCTCTGTGAC
721   ---------+---------+---------+---------+---------+---------+   780

      S  C  V  E  P  W  Q  C  N  C  E  T  N  W  G  G  L  L  C  D

      AAAGACCTGAACTACTGTGGCAGCCACCACCCCTGCACCAACGGAGGCACGTGCATCAAC
781   ---------+---------+---------+---------+---------+---------+   840

      K  D  L  N  Y  C  G  S  H  H  P  C  T  N  G  G  T  C  I  N

      GCCGAGCCTGACCAGTACCGCTGCACCTGCCCTGACGGCTACTCGGGCAGGAACTGTGAG
841   ---------+---------+---------+---------+---------+---------+   900

      A  E  P  D  Q  Y  R  C  T  C  P  D  G  Y  S  G  R  N  C  E

      AAGGCTGAGCACGCCTGCACCTCCAACCCGTGTGCCAACGGGGGCTCTTGCCATGAGGTG
901   ---------+---------+---------+---------+---------+---------+   960

      K  A  E  H  A  C  T  S  N  P  C  A  N  G  G  S  C  H  E  V

      CCGTCCGGCTTCGAATGCCACTGCCCATCGGGCTGGAGCGGGCCCACCTGTGCCCTTGAC
961   ---------+---------+---------+---------+---------+---------+  1020

      P  S  G  F  E  C  H  C  P  S  G  W  S  G  P  T  C  A  L  D

      ATCGATGAGTGTGCTTCGAACCCGTGTGCGGCCGGTGGCACCTGTGTGGACCAGGTGGAC
1021  ---------+---------+---------+---------+---------+---------+  1080

      I  D  E  C  A  S  N  P  C  A  A  G  G  T  C  V  D  Q  V  D

      GGCTTTGAGTGCATCTGCCCCGAGCAGTGGGTGGGGGCCACCTGCCAGCTGGACGTCAAC
1081  ---------+---------+---------+---------+---------+---------+  1140

      G  F  E  C  I  C  P  E  Q  W  V  G  A  T  C  Q  L  D  V  N

      GACTGTCGCGGGCAGTGTCAGCATGGGGGCACCTGCAAGGACCTGGTGAACGGGTACCAG
1141  ---------+---------+---------+---------+---------+---------+  1200

      D  C  R  G  Q  C  Q  H  G  G  T  C  K  D  L  V  N  G  Y  Q
```

FIG. 1B-2

```
      TGTGTGTGCCCACGGGGCTTCGGAGGCCGGCATTGCGAGCTGGAACGAGACAAGTGTGCC
1201  ---------+---------+---------+---------+---------+---------+  1260

      C  V  C  P  R  G  F  G  G  R  H  C  E  L  E  R  D  K  C  A

      AGCAGCCCCTGCCACAGCGGCGGCCTCTGCGAGGACCTGGCCGACGGCTTCCACTGCCAC
1261  ---------+---------+---------+---------+---------+---------+  1320

      S  S  P  C  H  S  G  G  L  C  E  D  L  A  D  G  F  H  C  H

      TGCCCCCAGGGCTTCTCCGGGCCTCTCTGTGAGGTGGATGTCGACCTTTGTGAGCCAAGC
1321  ---------+---------+---------+---------+---------+---------+  1380

      C  P  Q  G  F  S  G  P  L  C  E  V  D  V  D  L  C  E  P  S

      CCCTGCCGGAACGGCGCTCGCTGCTATAACCTGGAGGGTGACTATTACTGCGCCTGCCCT
1380  ---------+---------+---------+---------+---------+---------+  1440

      P  C  R  N  G  A  R  C  Y  N  L  E  G  D  Y  Y  C  A  C  P

      GATGACTTTGGTGGCAAGAACTGCTCCGTGCCCCGCGAGCCGTGCTGGCGGGGCCTGCAG
1441  ---------+---------+---------+---------+---------+---------+  1500

      D  D  F  G  G  K  N  C  S  V  P  R  E  P  C  W  R  G  L  Q

      AGTGATCGATGGCTGCGGGTCAGACGCGGGGCCTGGGATGCCTGGCACAGCACGTCCGGC
1501  ---------+---------+---------+---------+---------+---------+  1560

      S  D  R  W  L  R  V  R  R  G  A  W  D  A  W  H  S  T  S  G

      GTGTGTGGCCCCCATGGACGCTGCGTCAGCCAGCCAGGGGGCAACTTTTCCTGCATCTGT
1561  ---------+---------+---------+---------+---------+---------+  1620

      V  C  G  P  H  G  R  C  V  S  Q  P  G  G  N  F  S  C  I  C

      GACAGTGGCTTTACTGGCACCTACTGCCATGAGAACATTGACGACTGCCTGGGCCAGCCC
1621  ---------+---------+---------+---------+---------+---------+  1680

      D  S  G  F  T  G  T  Y  C  H  E  N  I  D  D  C  L  G  Q  P

      TGCCGCAATGGGGGCACATGCATCGATGAGGTGGACGCCTTCCGCTGCTTCTGCCCCAGC
1681  ---------+---------+---------+---------+---------+---------+  1740

      C  R  N  G  G  T  C  I  D  E  V  D  A  F  R  C  F  C  P  S

      GGCTGGGAGGGCGAGCTCTGCGACACCAATCCCAACGACTGCCTTCCCGATCCCTGCCAC
1741  ---------+---------+---------+---------+---------+---------+  1800

      G  W  E  G  E  L  C  D  T  N  P  N  D  C  L  P  D  P  C  H
```

FIG. 1B-3

```
                AGCCGCGGCCGCTGCTACGACCTGGTCAATGACTTCTACTGTGCGTGCGACGACGGCTGG
1801  --------+---------+---------+---------+---------+---------+---------+  1860

                S  R  G  R  C  Y  D  L  V  N  D  F  Y  C  A  C  D  D  G  W

                AAGGGCAAGACCTGCCACTCACGCGAGTTCCAGTGCGATGCCTACACCTGCAGCAACGGT
1861  --------+---------+---------+---------+---------+---------+---------+  1920

                K  G  K  T  C  H  S  R  E  F  Q  C  D  A  Y  T  C  S  N  G

                GGCACCTGCTACGACAGCGGCGACACCTTCCGCTGCGCCTGCCCCCCCGGCTGGAAGGGC
1921  --------+---------+---------+---------+---------+---------+---------+  1980

                G  T  C  Y  D  S  G  D  T  F  R  C  A  C  P  P  G  W  K  G

                AGCACCTGCGCCGTCGCCAAGAACAGCAGCTGCCTGCCCAACCCCTGTGTGAATGGTGGC
1981  --------+---------+---------+---------+---------+---------+---------+  2040

                S  T  C  A  V  A  K  N  S  S  C  L  P  N  P  C  V  N  G  G

                ACCTGCGTGGGCAGCGGGGCCTCCTTCTCCTGCATCTGCCGGGACGGCTGGGAGGGTCGT
2041  --------+---------+---------+---------+---------+---------+---------+  2100

                T  C  V  G  S  G  A  S  F  S  C  I  C  R  D  G  W  E  G  R

                ACTTGCACTCACAATACCAACGACTGCAACCCTCTGCCTTGCTACAATGGTGGCATCTGT
2101  --------+---------+---------+---------+---------+---------+---------+  2160

                T  C  T  H  N  T  N  D  C  N  P  L  P  C  Y  N  G  G  I  C

                GTTGACGGCGTCAACTGGTTCCGCTGCGAGTGTGCACCTGGCTTCGCGGGGCCTGACTGC
2161  --------+---------+---------+---------+---------+---------+---------+  2220

                V  D  G  V  N  W  F  R  C  E  C  A  P  G  F  A  G  P  D  C

                CGCATCAACATCGACGAGTGCCAGTCCTCGCCCTGTGCCTACGGGGCCACGTGTGTGGAT
2221  --------+---------+---------+---------+---------+---------+---------+  2280

                R  I  N  I  D  E  C  Q  S  S  P  C  A  Y  G  A  T  C  V  D

                GAGATCAACGGGTATCGCTGTAGCTGCCCACCCGGCCGAGCCGGCCCCCGGTGCCAGGAA
2281  --------+---------+---------+---------+---------+---------+---------+  2340

                E  I  N  G  Y  R  C  S  C  P  P  G  R  A  G  P  R  C  Q  E

                GTGATCGGGTTCGGGAGATCCTGCTGGTCCCGGGGCACTCCGTTCCCACACGGAAGCTCC
2341  --------+---------+---------+---------+---------+---------+---------+  2400

                V  I  G  F  G  R  S  C  W  S  R  G  T  P  F  P  H  G  S  S
```

FIG. 1B-4

```
                TGGGTGGAAGACTGCAACAGCTGCCGCTGCCTGGATGGCCGCCGTGACTGCAGCAAGGTG
2401  --------+---------+---------+---------+---------+---------+---------+  2460
                W  V  E  D  C  N  S  C  R  C  L  D  G  R  R  D  C  S  K  V

                TGGTGCGGATGGAAGCCTTGTCTGCTGGCCGGCCAGCCCGAGGCCCTGAGCGCCCAGTGC
2461  --------+---------+---------+---------+---------+---------+---------+  2520
                W  C  G  W  K  P  C  L  L  A  G  Q  P  E  A  L  S  A  Q  C

                CCACTGGGGCAAAGGTGCCTGGAGAAGGCCCCAGGCCAGTGTCTGCGACCACCCTGTGAG
2521  --------+---------+---------+---------+---------+---------+---------+  2580
                P  L  G  Q  R  C  L  E  K  A  P  G  Q  C  L  R  P  P  C  E

                GCCTGGGGGGAGTGCGGCGCAGAAGAGCCACCGAGCACCCCCTGCCTGCCACGCTCCGGC
2581  --------+---------+---------+---------+---------+---------+---------+  2640
                A  W  G  E  C  G  A  E  E  P  P  S  T  P  C  L  P  R  S  G

                CACCTGGACAATAACTGTGCCCGCCTCACCTTGCATTTCAACCGTGACCACGTGCCCCAG
2641  --------+---------+---------+---------+---------+---------+---------+  2700
                H  L  D  N  N  C  A  R  L  T  L  H  F  N  R  D  H  V  P  Q

                GGCACCACGGTGGGCGCCATTTGCTCCGGGATCCGCTCCCTGCCAGCCACAAGGGCTGTG
2701  --------+---------+---------+---------+---------+---------+---------+  2760
                G  T  T  V  G  A  I  C  S  G  I  R  S  L  P  A  T  R  A  V

                GCACGGGACCGCCTGCTGGTGTTGCTTTGCGACCGGGCGTCCTCGGGGGCCAGTGCCGTG
2761  --------+---------+---------+---------+---------+---------+---------+  2820
                A  R  D  R  L  L  V  L  L  C  D  R  A  S  S  G  A  S  A  V

                GAGGTGGCCGTGTCCTTCAGCCCTGCCAGGGACCTGCCTGACAGCAGCCTGATCCAGGGC
2821  --------+---------+---------+---------+---------+---------+---------+  2880
                E  V  A  V  S  F  S  P  A  R  D  L  P  D  S  S  L  I  Q  G

                GCGGCCCACGCCATCGTGGCCGCCATCACCCAGCGGGGGAACAGCTCACTGCTCCTGGCT
2881  --------+---------+---------+---------+---------+---------+---------+  2940
                A  A  H  A  I  V  A  A  I  T  Q  R  G  N  S  S  L  L  L  A

                GTCACCGAGGTCAAGGTGGAGACGGTTGTTACGGGCGGCTCTTCCACAGGTCTGCTGGTG
2941  --------+---------+---------+---------+---------+---------+---------+  3000
```

FIG. 1B-5

```
      V  T  E  V  K  V  E  T  V  V  T  G  G  S  S  T  G  L  L  V

      CCTGTGCTGTGTGGTGCCTTCAGCGTGCTGTGGCTGGCGTGCGTGGTCCTGTGCGTGTGG
3001  ---------+---------+---------+---------+---------+---------+---------  3060

      P  V  L  C  G  A  F  S  V  L  W  L  A  C  V  V  L  C  V  W

      TGGACACGCAAGCGCAGGAAAGAGCGGGAGAGGAGCCGGCTGCCGCGGGAGGAGAGCGCC
3061  ---------+---------+---------+---------+---------+---------+---------  3120

      W  T  R  K  R  R  K  E  R  E  R  S  R  L  P  R  E  E  S  A

      AACAACCAGTGGGCCCCGCTCAACCCCATCCGCAACCCCATCGAGCGGCCGGGGGGCCAC
3121  ---------+---------+---------+---------+---------+---------+---------  3180

      N  N  Q  W  A  P  L  N  P  I  R  N  P  I  E  R  P  G  G  H

      AAGGACGTGCTCTACCAGTGCAAGAACTTCACGCCGCCGCCGCGCAGGGCGGACGAGGCG
3181  ---------+---------+---------+---------+---------+---------+---------  3240

      K  D  V  L  Y  Q  C  K  N  F  T  P  P  P  R  R  A  D  E  A

      CTGCCCGGGCCGGCGCGCCACGCGGCCGTCAGGGAGGATGAGGAGGACGAGGATCTGGGC
3241  ---------+---------+---------+---------+---------+---------+---------  3300

      L  P  G  P  A  R  H  A  A  V  R  E  D  E  E  D  E  D  L  G

      CGCGGTGAGGAGGACTCCCTGGAGGCGGAGAAGTTCCTCTCACACAAATTCACCAAAGAT
3301  ---------+---------+---------+---------+---------+---------+---------  3360

      R  G  E  E  D  S  L  E  A  E  K  F  L  S  H  K  F  T  K  D

      CCTGGCCGCTCGCCGGGGAGGCCCGCCCACTGGCCTCAGGCCCCAAAGTGGACAACCGCG
3361  ---------+---------+---------+---------+---------+---------+---------  3420

      P  G  R  S  P  G  R  P  A  H  W  P  Q  A  P  K  W  T  T  A

      CGGTCAGGAGCATCAATGAGGCCCTACGCCGGCAAGGAGTAGGGGCGGCTGCCAGCTGGG
3421  ---------+---------+---------+---------+---------+---------+---------  3480
      R  S  G  A  S  M  R  P  Y  A  G  K  E  *

      CCGGGACCCAGGGCCCTCGGTGGGAGCCATGCCGTCTGCCGGACCCGGAGGCCGAGGCCA
3481  ---------+---------+---------+---------+---------+---------+---------  3540

      TGTGCATAGTTTCTTTATTTTGTGTAAAAAAACCACCAAAAACAAAAACCAAATGTTTAT
3541  ---------+---------+---------+---------+---------+---------+---------  3600

      TTTCTACGTTTCTTTAACCTTGTATAAATTATTCAGTAACTGTCAGGCTGAAAACAATGG
3601  ---------+---------+---------+---------+---------+---------+---------  3660

      AGTATTCTCGGATAGTTGCTATTTTTGTAAAGTTTCCGTGCGTGGCACTCGCTGTATGAA
3661  ---------+---------+---------+---------+---------+---------+---------  3720
```

FIG. 1B-6

```
3721  AGGAGAGAGCAAAGGGTGTCTGCGTCGTCACCAAATCGTAGCGTTTGTTACCAGAGGTTG
      ---------+---------+---------+---------+---------+---------+ 3780
3781  TGCACTGTTTACAGAATCTTCCTTTTATTCCTCACTCGGGTTTCTCTGTGGCTCCAGGCC
      ---------+---------+---------+---------+---------+---------+ 3840
3841  AAAGTGCCGGTGAGACCCATGGCTGTGTTGGTGTGGCCCATGGCTGTTGGTGGGACCCGT
      ---------+---------+---------+---------+---------+---------+ 3900
3901  GGCTGATGGTGTGGCCTGTGGCTGTCGGTGGGACTCGTGGCTGTCAATGGGACCTGTGGC
      ---------+---------+---------+---------+---------+---------+ 3960
3961  TGTCGGTGGGACCTACGGTGGTCGGTGGGACCCTGGTTATTGATGTGGCCCTGGCTGCCG
      ---------+---------+---------+---------+---------+---------+ 4020
4021  GCACGGCCCGTGGCTGTTGACGCACCTGTGGTTGTTAGTGGGGCCTGAGGTCATCGGCGT
      ---------+---------+---------+---------+---------+---------+ 4080
4081  GGCCCAAGGCCGGCAGGTCAACCTCGCGCTTGCTGGCCAGTCCACCCTGCCTGCCGTCTG
      ---------+---------+---------+---------+---------+---------+ 4140
4141  TGCTTCCTCCTGCCCAGAACGCCCGCTCCAGCGATCTCTCCACTGTGCTTTCAGAAGTGC
      ---------+---------+---------+---------+---------+---------+ 4200
4201  CCTTCCTGCTGCGAAGTTCTCCCATCCTGGGACGGCGGCAGTATTGAAGCTCGTGACAAG
      ---------+---------+---------+---------+---------+---------+ 4260
4261  TGCCTTCACACAGAACCCTCGGAACTGTCCACGCGTTCCGTGGGAACAAGGGGTT
      ---------+---------+---------+---------+---------+-----  4315
```

FIG. 1B-7

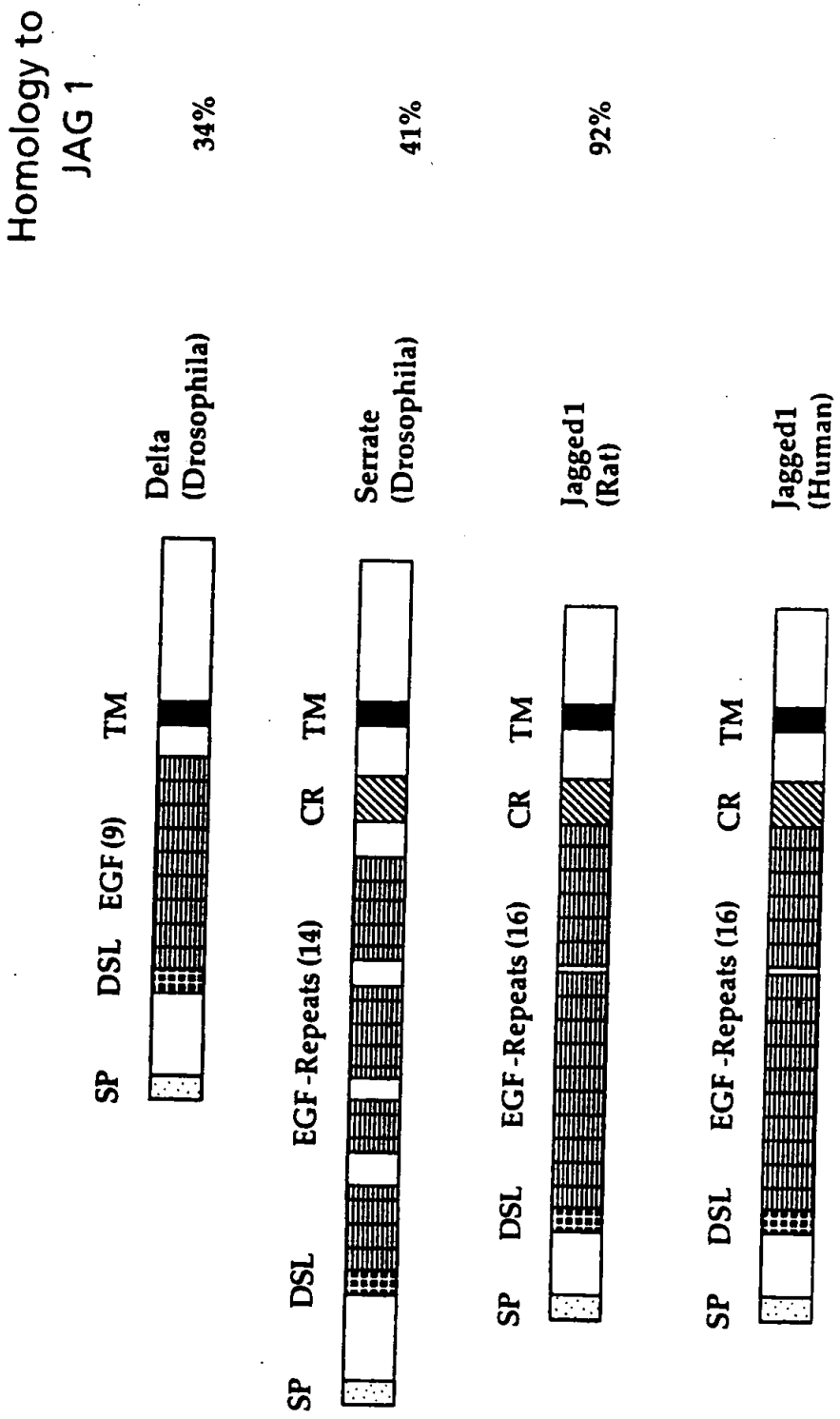


FIG. 1C

```
hjg1     1 MRSPRTRGRSGRPLSLLLALLCALRAKVCGASGQFELEILSMQNVNGELQNGNCCGGARN 60
rjg          ::::P::::P::::::::::::::::::::::::::::::::::::::::::AEPGT
hjg1    61 PGDR-KCTRDECDTYFKVCLKEYQSRVTAGGPCSFGSGSTPVIGGNTFNLKASRGNDRNR 120
rjg          LVRPY::::::::::::::::::::::::C:::::::::::::::::::::::::::::
hjg1   121 IVLPFSFAWPRSYTLLVEAWDSSNDTVQPDSIIEKASHSGMINPSRQWQTLKQNTGVAHF 180
rjg          ::::::::::::::::::::G::::I::::::::::::::::::::::::::::I:::
hjg1   181 EYQIRVTCDDYYYGFGCNKFCRPRDDFFGHYACDQNGNKTCMEGWMGPECNRAICRQGCS 240
rjg          ::::::::::H:::P::::::::::::::::::::::::::::::::::::K::::::::
hjg1   241 PKHGSCKLPGDCRCQYGWQGLYCDKCIPHPGCVHGICNEPWQCLCETNWGGQLCDKDLNY 300
rjg          ::::::::::::::::::::::::::::::::::T::::::::::::::::::::::::
hjg1   301 CGTHQPCLNGGTCSNTGPDKYQCSCPEGYSGPNCEIAEHACLSDPCHNRGSCKETSLGFE 360
rjg          :::::::::R::::::::::::::::::::::::::::::::::::::::::::::S:::
hjg1   361 CECSPGWTGPTCSTNIDDCSPNNCSHGGTCQDLVNGFKCVCPPQWTGKTCQLDANECEAK 420
rjg          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
hjg1   421 PCVNAKSCKNLIASYYCDCLPGWMGQNCDININDCLGQCQNDASCRDLVNGYRCICPPGY 480
rjg          :::::R:::V::::::::::::::::::::::::::::::::::::::::::::::::::
hjg1   481 AGDHCERDIDECASNPCLNGGHCQNEINRFQCLCPTGFSGNLCQLDIDYCEPNPCQNGAQ 540
rjg          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
hjg1   541 CYNRASDYFCKCPEDYEGKNCSHLKDHCRTTPCEVIDSCTVAMASNDTPEGVRYISSNVC 600
rjg          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
hjg1   601 GPHGKCKSQSGGKFTCDCNKGFTGTYCHENINDCESNPCRNGGTCIDGVNSYKCICSDGW 660
rjg          ::::::::E::::::::::::::::::::ECG:::T::::::::::::::::::::::
hjg1   661 EGAYCETNINDCSQNPCHNGGTCRDLVNDFYCDCKNGWKGKTCHSRDSQCDEATCNNGGT 720
rjg          :::H::N::::::::::Y::::::::::::::::::::::::::::::::::::::::::
hjg1   721 CYDEGDAFKCMCPGGWEGTTCNIARNSSCLPNPCHNGGTCVVNGESFTCVCKEGWEGPIC 780
rjg          ::::V:T::::::::::::::::::::::::CP::::::::::D::::::::::::::
hjg1   781 AQNTNDCSPHPCYNSGTCVDGDNWYRCECAPGFAGPDCRININECQSSPCAFGATCVDEI 840
rjg          T::::::::::::::::::::::Q::::::::::::::::::::::::::::::::::
hjg1   841 NGYRCVCPPGHSGAKCQEVSGRPCITMGSVIPDGAKWDDDCNTCQCLNGRIACSKVWCGP 900
rjg          :::Q:I::::::::::H::C::S:::::R::L::::::::::-:::::V:S:::::::
hjg1   901 RPCLLHKGHSECPSGQSCIPILDDQCFVHPCTGVGECRSSSLQPVKTKCTSDSYYQDNCA 960
rjg          :::R:::::G:::N::::::V:::::::R::::A::::::::::::::::::::::::::
hjg1   961 NITFTFNKEMMSPGLTTEHICSELRNLNILKNVSAEYSIYIACEPSPSANNEIHVAISAE 1020
rjg          ::::::::::::::::::::::::::::::::::::::::::::::::L::::::::::::
hjg1  1021 DIRDDGNPIKEITDKIIDLVSKRDGNSSLIAAVAEVRVQRRPLKNRTDFLVPLLSSVLTV 1080
rjg          ::::::::V::::::::::::::::::::::::::::::::::::::::::::::::::::
hjg1  1081 AWICCLVTAFYWCLRK-RRKPGSHTHSASEDNTTNNVREQLNQIKNPIEKHGANTVPIKD 1140
rjg          ::V::::::::::V::R::::S::::::P::::::::::D::::::::::::::::::::
hjg1  1141 YENKNSKMSKIRTHNSEVEEDDMDKHQQKARFAKQPAYTLVDREEKPPNGTPTKHPNWIN 1200
rjg          ::::::::::::::::::::::::::::V::::::V::::::::::V:QR::::::::::
hjg1  1201 KQDNRDLESAQSLNRMEYIV 1220
rjg          ::::::::::::::::::::
```

FIG. 2A

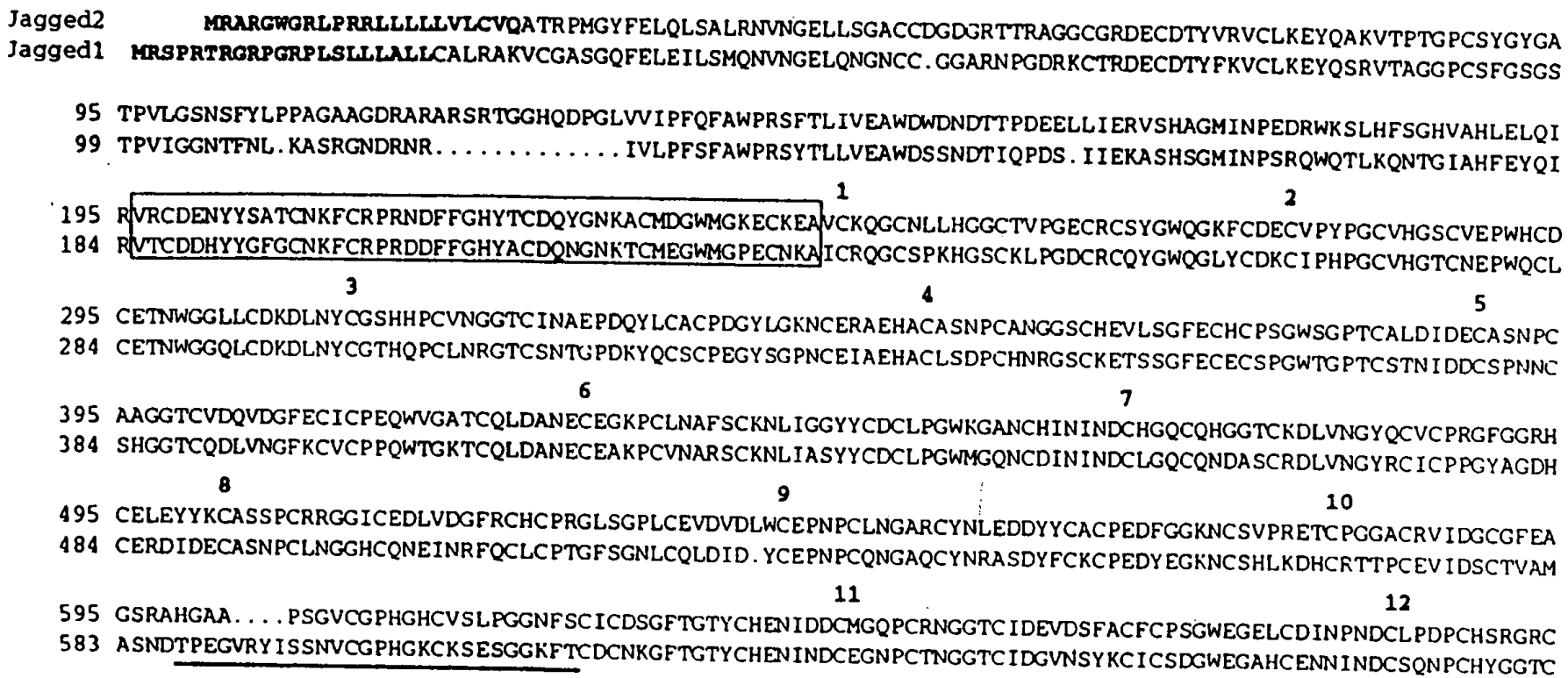


FIG. 2B-1

```
                                    13                                  14
691 YDLVNDFYCVCDDGWKDKTCHSREFQCDAYTCSNGGTCYDSGDTFRCACPPGWKGSTCTIAKNSSCVPNPCVNGGTCVGSGDSFSCICRDGWEGRTCTHN
683 RDLVNDFYCDCKNGWKGKTCHSRDSQCDEATCNNGGTCYDEVDTFKCMCPGGWEGTTCNIARNSSCLPNPCHNGGTCVVNGDSFTCVCKEGWEGPICTQN

      15                                    16
791 TNDCNPLPCYNGGICVDGVNWFRCECAPGFAGPDCRININDECQSSPCAYGATCVDEINGYRCSCPPGRSGPRCQEVVIFTRPCWSRGVSFPHGSSWVEDC
783 TNDCSPHPCYNSGTCVDGDNWYRCECAPGFAGPDCRININECQSSPCAFGATCVDEINGYQCICPPGHSGAKCHEVS..GRSCITMGRVILDGAKWDDDC

891 NSCRCLDGHRDCSKVWCGWKPCLLSPQPSALSAQCPPGQQCREKAMGQCLQPPCENWGECTAEDPLPPSTPCLPRTTHLDNNCARLTLHFNRDQVPQGTT
881 NTCQCLNGRVACSKVWCGPRPCRLHKGH....GECPNGQSCIPVLDDQCFVRPCTGAGECRSSSLQPVKTKC.TSDSYYQDNCANITFTFNKEMMSPGLT

991 VGAICSGIRALPATRAAARDRLLLLLCDRASSGASAVEVAVSFSPARDLPDSSLIQSTAHAIVAAITQR.GNSSLLLAVTEVKVETVVMGGSSTGLLVPV
976 TEHICSELRNLNILKNVSAEYSIYIACEPSLSANNEIHVAISAEDIRD..DGNPVKEITDKIIDLVSKRDGNSSLIAAVAEVRVQRRPLKN.RTDFLVPL

1090 LCSVFSVLWLACMVICVWWTRKRRKERERSR...LPRDESANNQWAPLNPIRNPIERPGSSGLGTGGHKDVLYQCKNFTPPPRRAGEALPGPASHGAGGE
1073 LSSVLTVAWVCCLVTAFYWCVRKRRRKPSSHTHSAPEDNTTNNVREQLNQIKNPIEKHGANTVPIKD.....YENKNSKMSKIRTHNS.....

1187 DEEDEELSRGDGRLSRSREVPLTQIHQRPQLLPGKASLLAP..GPKVDNRAVRSTKDVRCAGRE  1248
1156 EVEEDDMDKHQQKVRFAKQPVYTLV.DREEKVPQRTPTKHPNWTNKQDNRDLESAQSLNRMEYIV 1219
```

FIG. 2B-2

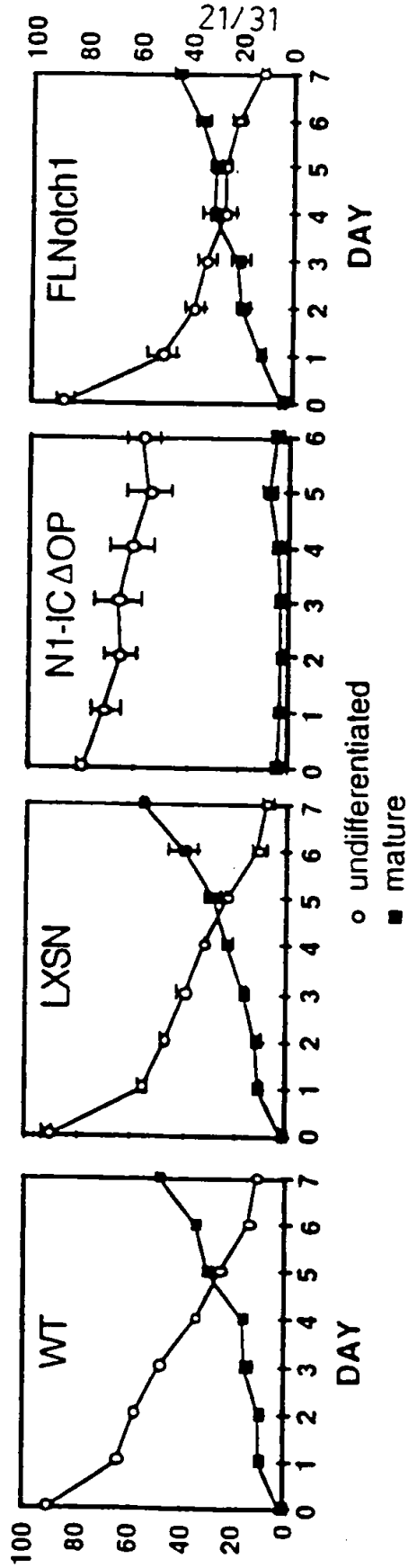


FIG. 3A

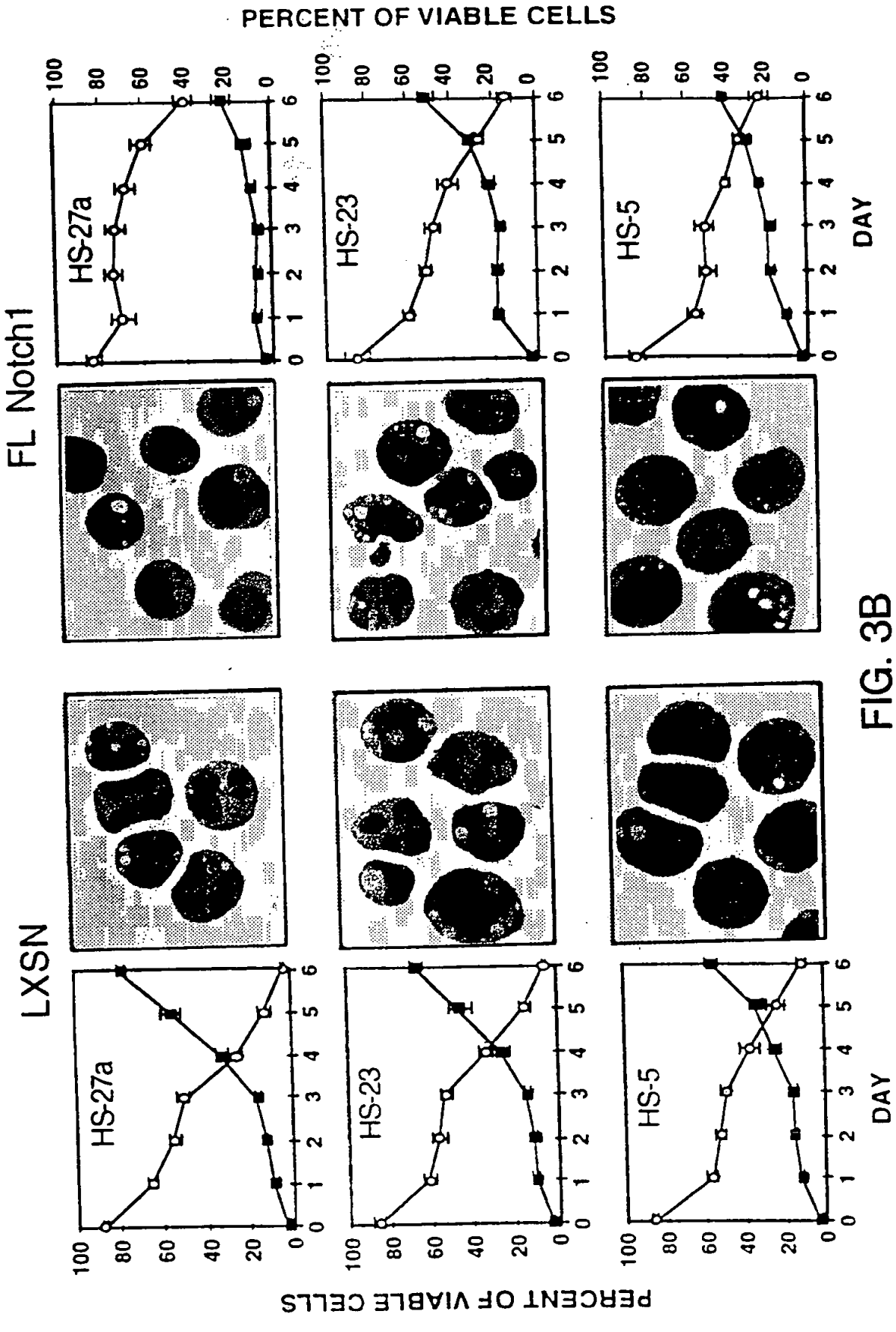

PERCENT OF VIABLE CELLS
FL Notch1
LXSN
HS-27a
HS-23
HS-5
DAY
0
20
40
60
80
100
0 1 2 3 4 5 6

FIG. 3B

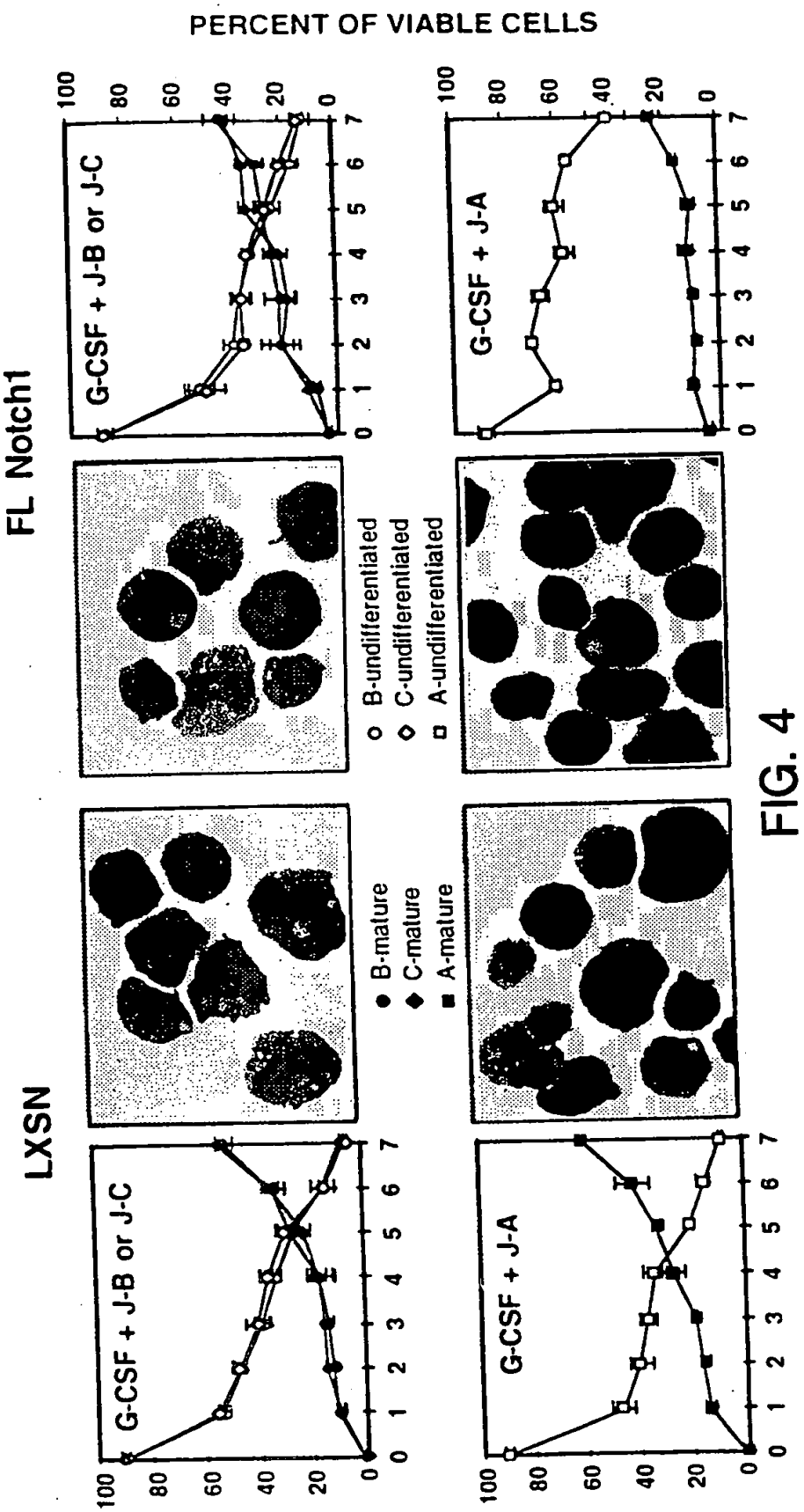
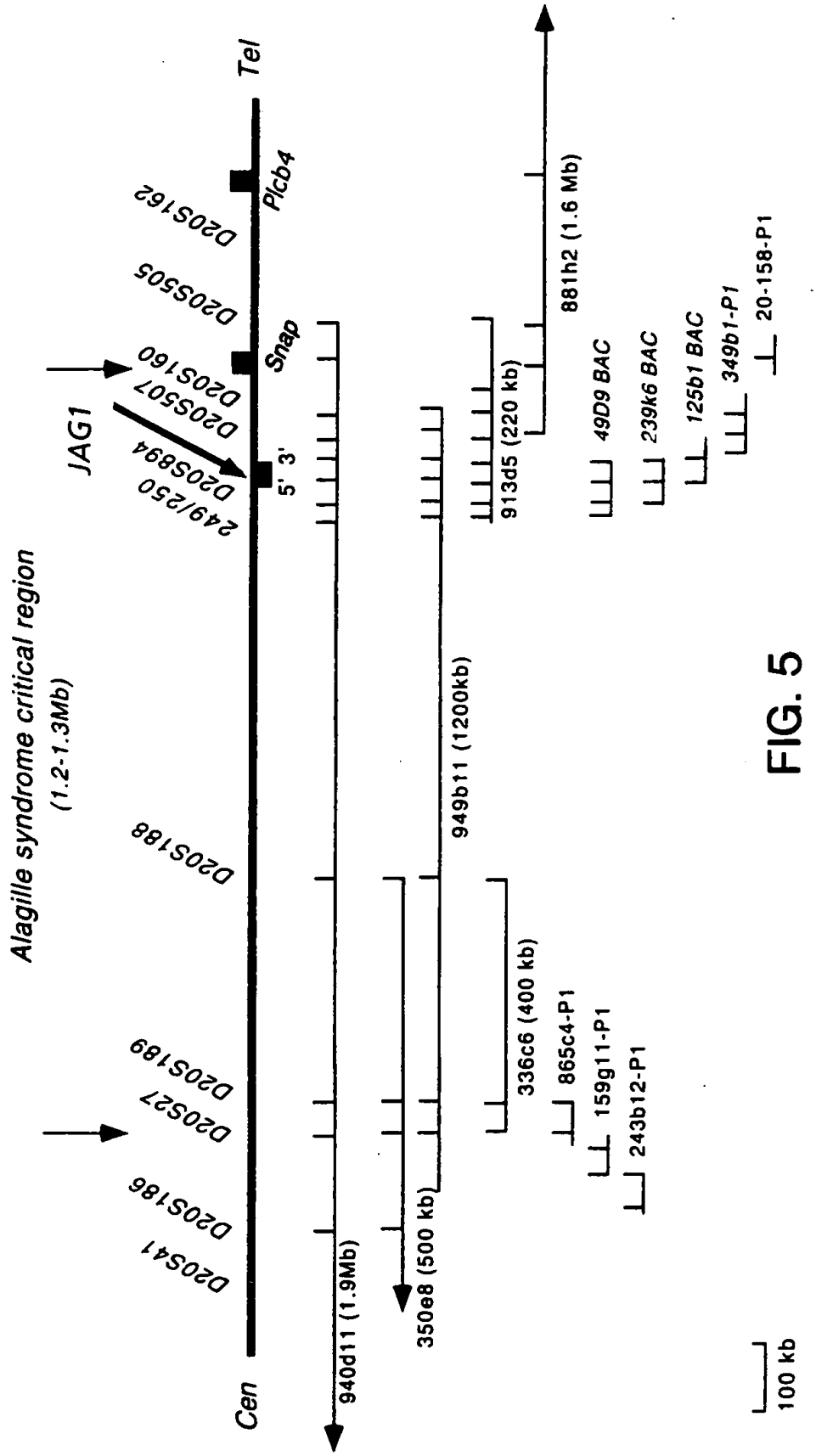


FIG. 4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(1.2-1.3Mb)
JAG1
Tel
Cen
Plcb4
Snap
5' 3'
D20S162
D20S505
D20S160
D20S507
D20S894
249/250
D20S188
D20S189
D20S27
D20S186
D20S41
940d11 (1.9Mb)
350e8 (500 kb)
949b11 (1200kb)
913d5 (220 kb)
881h2 (1.6 Mb)
336c6 (400 kb)
865c4-P1
159g11-P1
243b12-P1
49D9 BAC
239k6 BAC
125b1 BAC
349b1-P1
20-158-P1
100 kb

FIG. 5

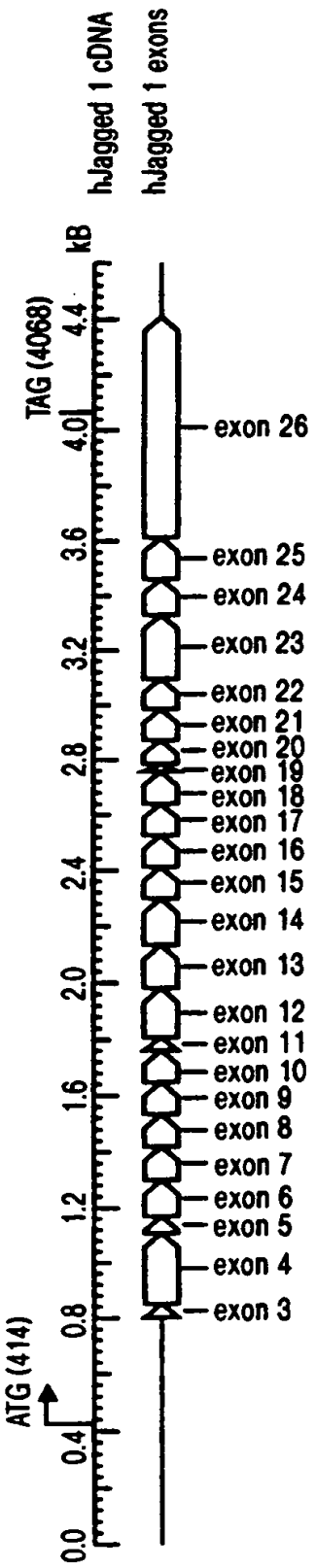


FIG. 6A

Table 1 • The Exon/Intron Boundary Sequences of *JAG1*

Exon Number	intron/EXON...EXON/intron	*JAG1* cDNA (bp)	Exon Length(bp)
Exons 1,2	not available		
Exon 3	gttcgtgcag/AGGTCCTACA(28)...GACACCGTTC/gtcagtatcg (29)	801-852	52
Exon 4	ttgtctctag/AACCTGACAG(30) ...TGTAACAGAG/gtatgtgtgt (31)	853-1107	255
Exon 5	ttttttacag/CTATTGCCG(32) ...GTGACTGCAG/g (33)	1108-1168	61
Exon 6	gtgtctccag/GTGCCAGTAT(34)...TGTGACAAAG/gtatggccct (35)	1169-1299	131
Exon 7	ttttttggcag/ATCTCAATTA(36) ...TGTGAAATTG/gtaagtggtc (37)	1300-1419	120
Exon 8	gtttttgcag/CTGAGCACGC(38)...TGCTCTACAA/gtaagtccaa (39)	1420-1533	114
Exon 9	tgttgaccag/ACATTGATGA(40) ...TGCCAGTTAG/gtaagaacat (41)	1534-1647	114
Exon 10	ctcctttcag/ATGCAAATGA(42) ...TGTGACATAA/gtgagtgact (43)	1648-1761	114
Exon 11	tatttttttag/ATATTAATGA (44) ...CTCCTGTCGG/gtatgtaaat (45)	1762-1808	47
Exon 12	cctcttctag/GATTTGGTTA(46) ...CCTCTGTCAG/gtgagtggtg (47)	1809-1982	174
Exon 13	atatttgcag/CTGGACATCG(48) ....CCCTGTGAAG/gtacctccct (49)	1983-2133	151
Exon 14	tatcttctag/TGATTGACAG(50) ...TGCCATGAAA/gtaagactcc (51)	2134-2298	165
Exon 15	tgtttcatag/ATATTAATGA(52) ...TGTGAAACCA/aagagtgtgc (53)	2299-2412	114
Exon 16	ttgattctag/ATATTAATGA(54) ....GGCCACTCAC/gtaagtggta (55)	2413-2526	114
Exon 17	tttcctccag/GTGACAGTCA(56) ...TGTAACATAG/gtaacttttat (57)	2527-2640	114
Exon 18	tcttttatag/CCCGAAACAG(58) ...TGTGCTCAGA/gtgagtgtcc (59)	2641-2757	117
Exon 19	ttctttgcag/ATACCAATGA(60) ....CTCATCCCTG/gtaagtgtga (61)	2758-2785	28
Exon 20	gctttcttag/TTACAACAGC(62) ....TGCAGAATAA/gtaaggactg (63)	2786-2871	86
Exon 21	tttctcctag/ACATCAATGA(64) ....TGCCAGGAAG/gtatgtgtgc (65)	2872-2985	114
Exon 22	cacctgtcag/TTTCAGGGAG(66) ....CTGCTCAAAG/gtaggacatg(67)	2986-3095	110
Exon 23	ttttctccag/GTCTGGTGTG(68) ....GATGTCACCA/gtatgtaaca (69)	3096-3329	234
Exon 24	atcgttttag/GGTCTTACTA(70) ....TGTGGCCATT/gtaagtataa (71)	3330-3461	132
Exon 25	gtttttccag/TCTGCTGAAG(72) ....AACAGAACAG/gtaggtgtca(73)	3462-3612	151
Exon 26	tgccttacag/ATTTCCTTGT(74)...	3613-4404+	792+

FIG. 6B

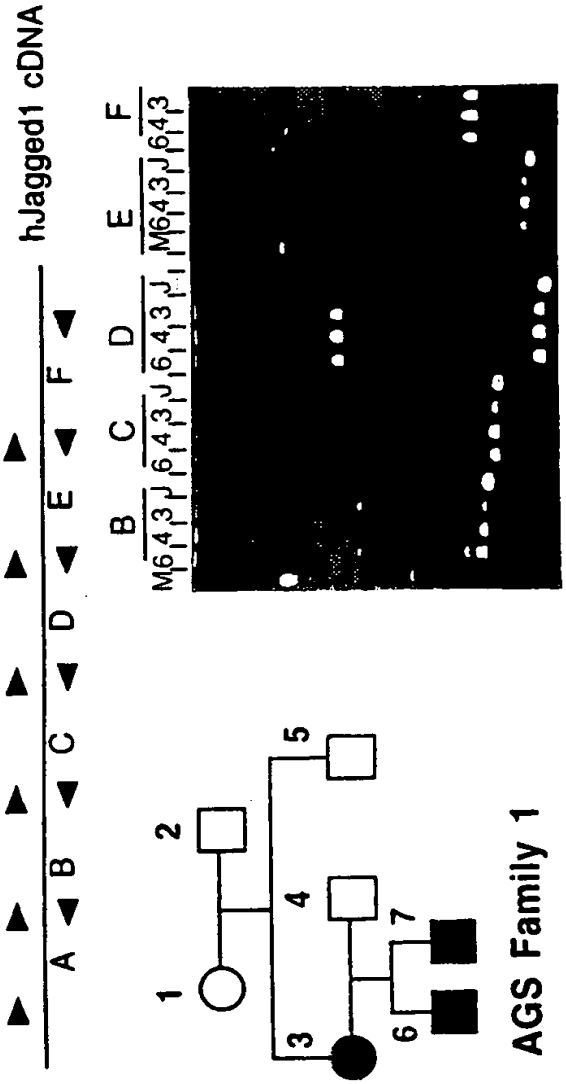
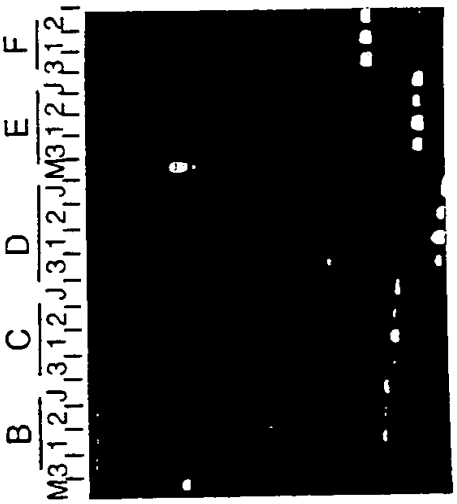
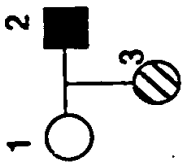


FIG. 7A

AGS Family 1

FIG. 7B





AGS Family 2

FIG. 7C

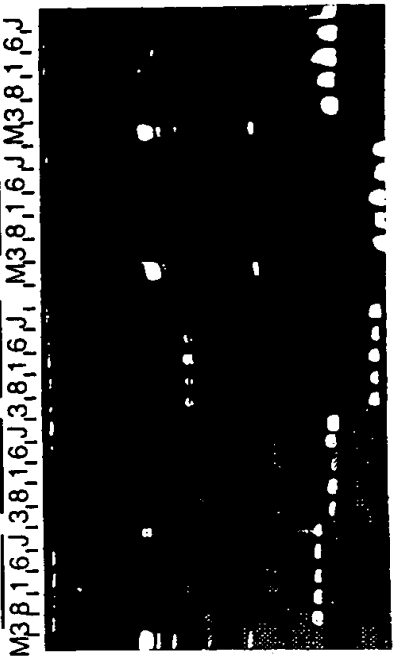
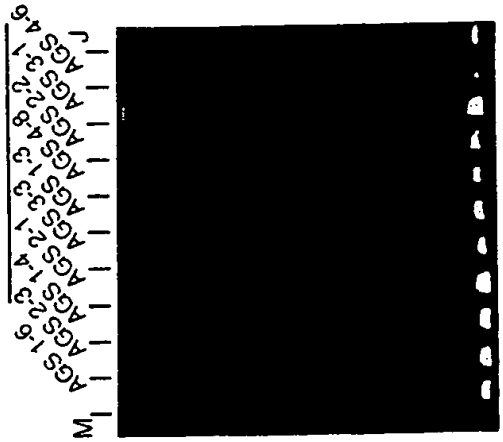
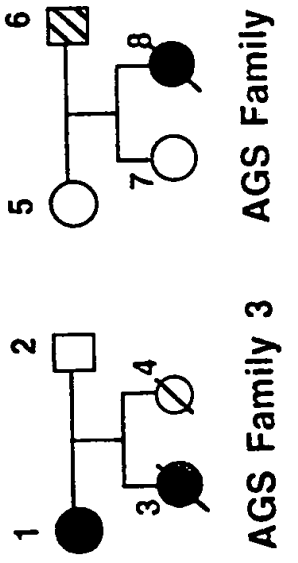


FIG. 7F





AGS Family 3 AGS Family 4

FIG. 7D

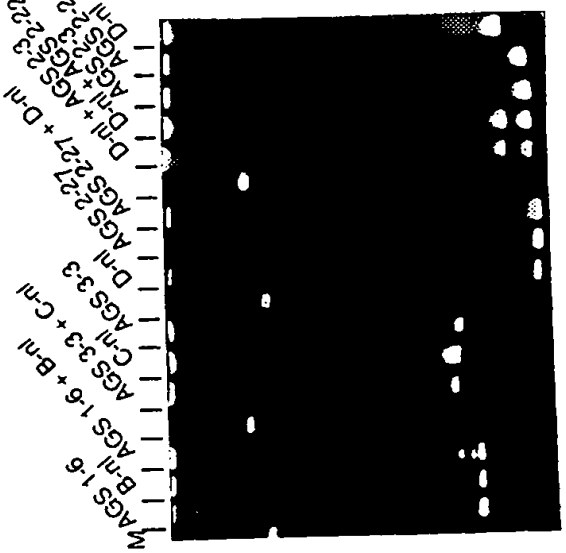
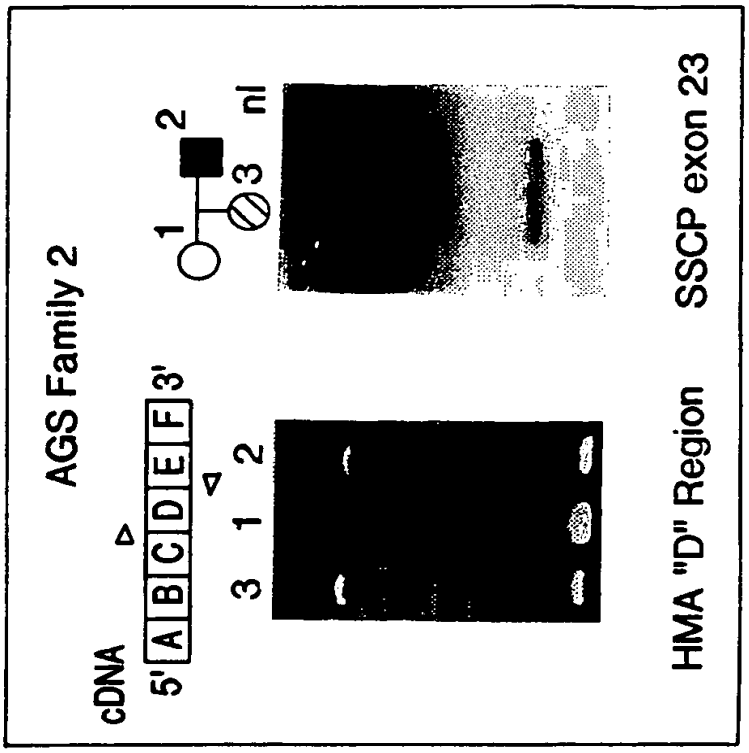


FIG. 7E



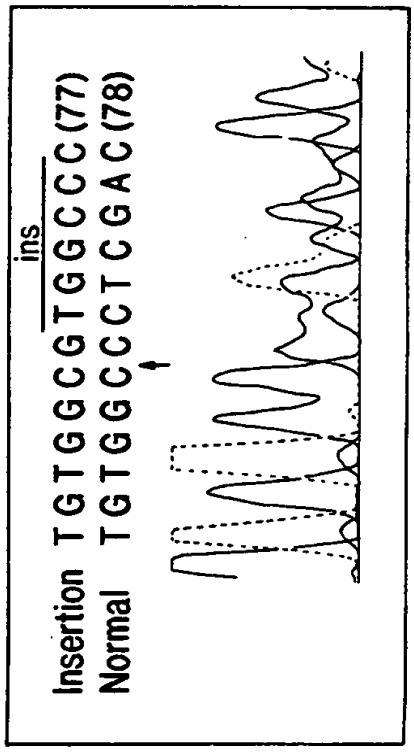
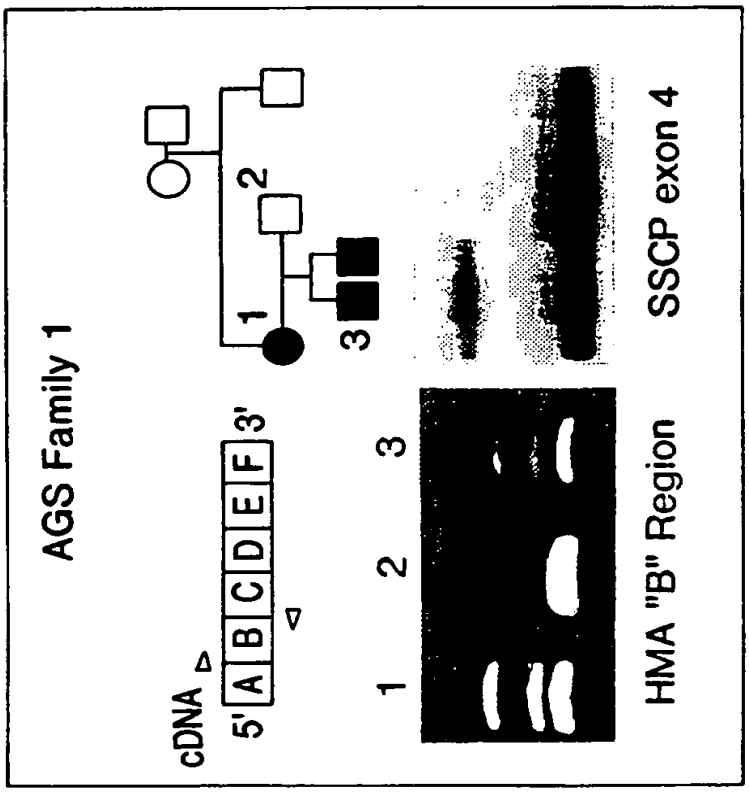


FIG. 8B



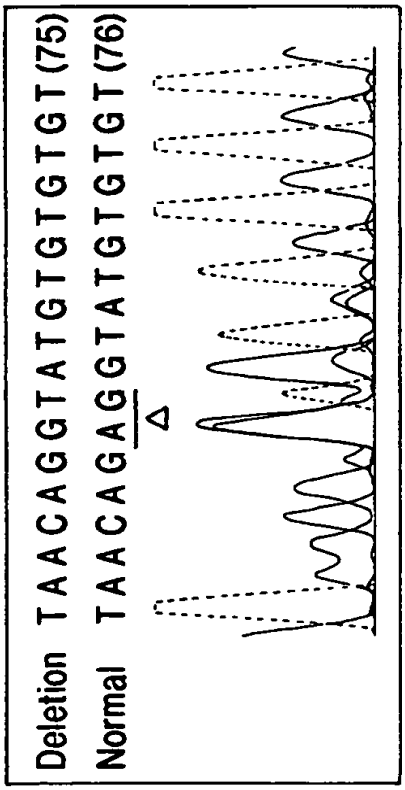


FIG. 8A

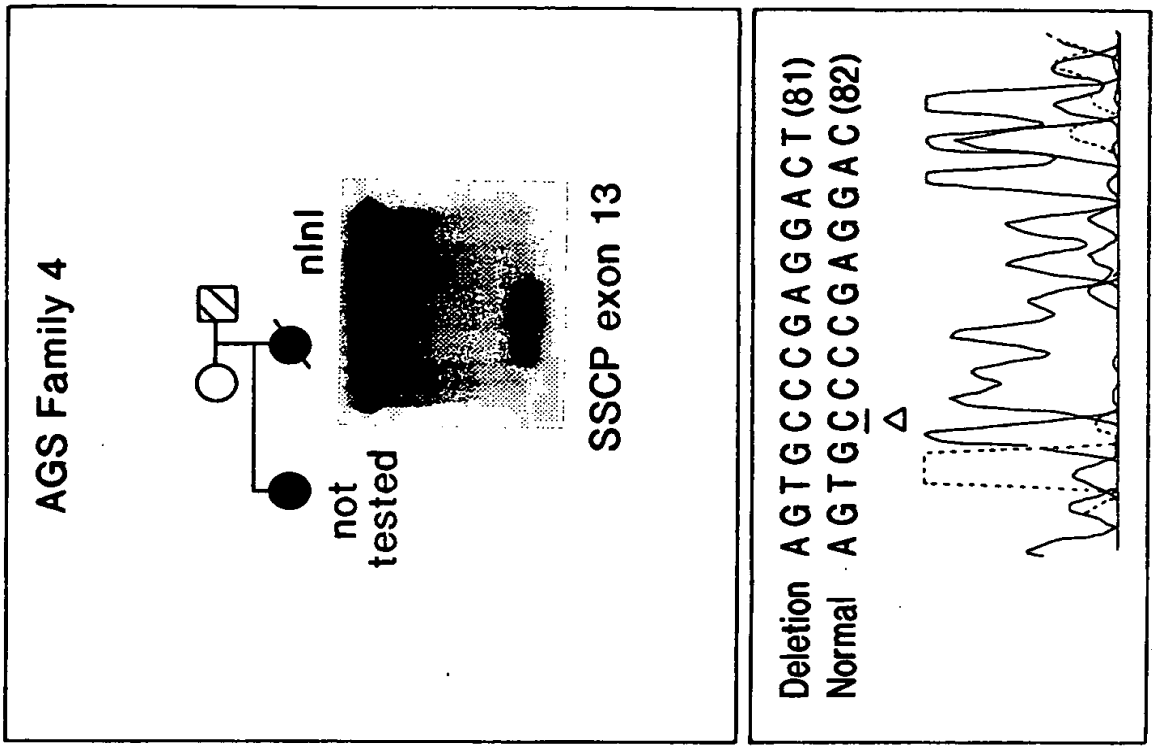

AGS Family 4
nl nl
not tested
SSCP exon 13
Deletion A G T G C C C G A G G A C T (81)
Normal A G T G C C C C G A G G A C (82)

FIG. 8D

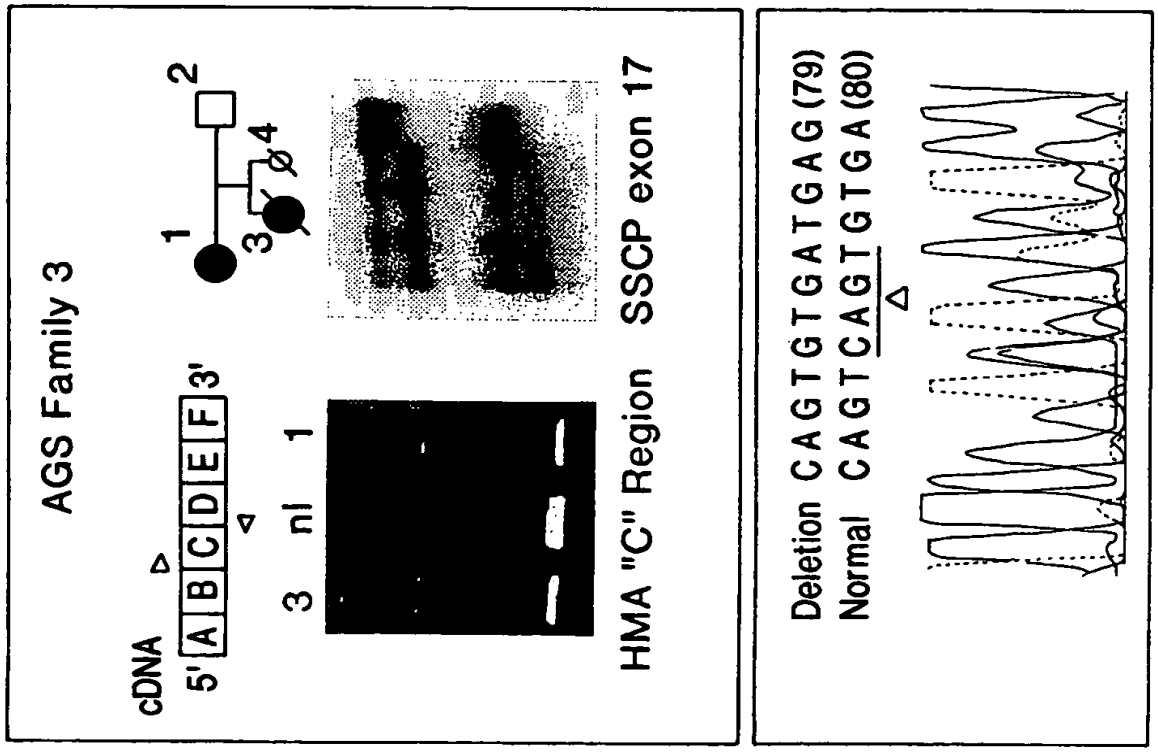

AGS Family 3
cDNA
5' A B C D E F 3'
1 2
3 4
3 nl 1
HMA "C" Region SSCP exon 17
Deletion C A G T G T G A T G A G (79)
Normal C A G T C A G T G T G A (80)

FIG. 8C

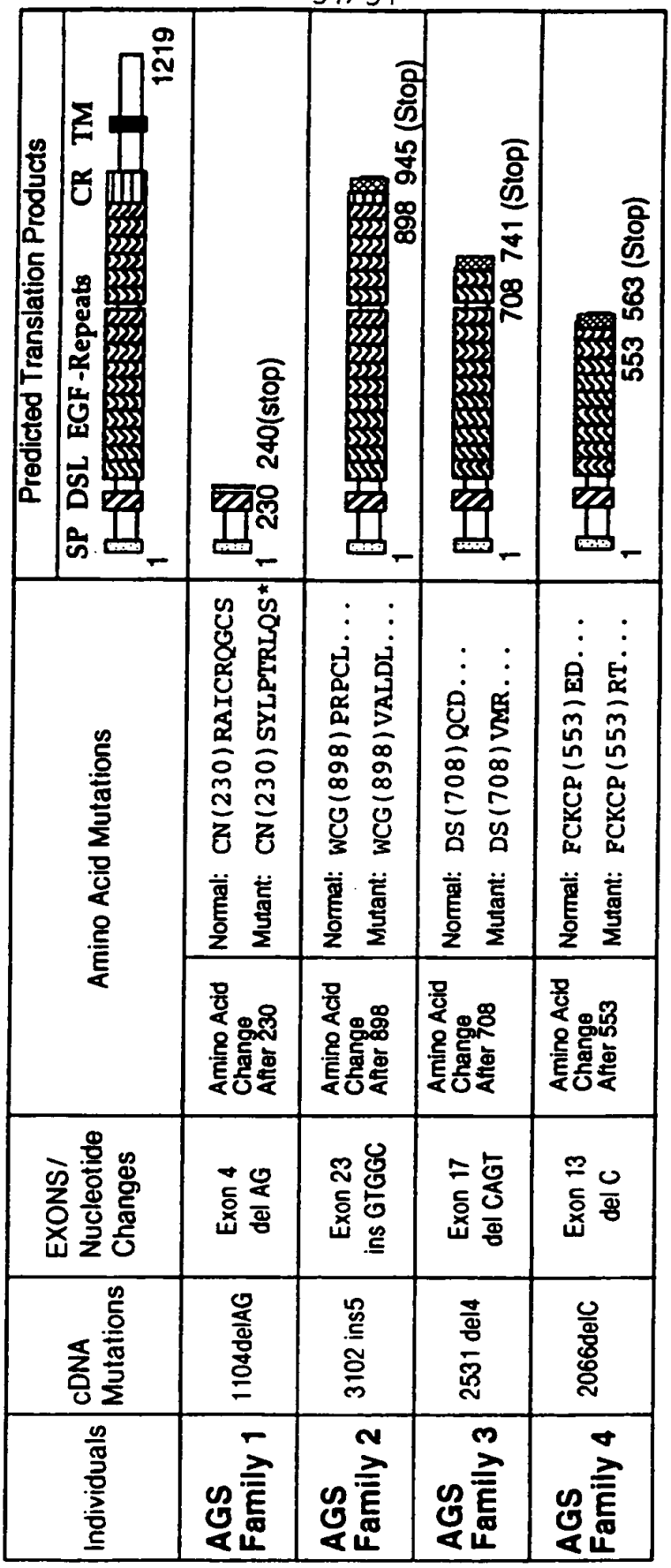


FIG. 9

INTERNATIONAL SEARCH REPORT

Int .tional Application No
PCT/US 98/13207

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 C07K14/47 C12Q1/68 C12N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	EP 0 861 894 A (ASAHI CHEMICAL IND) 2 September 1998 see the whole document	1,2, 4-17, 20-23
X	& WO 97 19172 A (ASAI CHEMICAL IND.) 29 May 1997 see abstract see page 62 - page 76	1,2, 4-17, 20-23
X	WO 96 27610 A (UNIV YALE ;IMP CANCER RES TECH (GB); ISH HOROWICZ DAVID (GB); HENR) 12 September 1996 see abstract see page 79, line 22 - page 86, line 24	1,2

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 January 1999	09/02/1999

Name and mailing address of the ISA	Authorized officer
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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LINDSELL ET AL.: "Jagged a mammalian ligand that activates Notch1" CELL, vol. 80, no. 6, 1995, pages 909-917, XP002090589	1,2
A	see the whole document	4-25
X,P	WO 97 45143 A (MONTESANO ROBERTO ;UNIV GENEVE (CH); PEPPER MICHAEL S (CH); MACIAG) 4 December 1997 see abstract see page 6, line 10 - page 9, line 30 see page 54 - page 61 see claims 1,2,48,49	1,2, 4-12, 14-17, 20-23
X,P	ODA T. ET AL.: "Mutations in the human Jagged1 gene are responsible for Alagille syndrome " NATURE GENETICS, vol. 16, no. 3, 1997, pages 235-242, XP002090587 us see the whole document	1,2, 26-30
X,P	ODA T. ET AL.: "Identification and cloning of the human homolog (JAG1) of the rat Jagged1 gene from the Alagille syndrome critical region at 20p12" GENOMICS, vol. 43, no. 3, 1 August 1997, pages 376-379, XP002090588 us see the whole document	1,2, 26-30
X,P	LI L. ET AL.: "Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1" NATURE GENETICS, vol. 16, no. 3, 1997, pages 243-251, XP002090590 us see the whole document	1,26-30

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INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/US 98/13207

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 26-31
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 26-31 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In itional Application No
PCT/US 98/13207

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0861894 A	02-09-1998	AU 7587696 A	11-06-1997
		CA 2236679 A	29-05-1997
		WO 9719172 A	29-05-1997
WO 9627610 A	12-09-1996	AU 5420296 A	23-09-1996
		CA 2214830 A	12-09-1996
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